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STUDIES WITH CIS-PLATINUM AND ITS EFFECTS ON THE BIOLOGICAL BEHAVIOR OF ^{67}Ga-CITRATE

by

THOMAS RITCHIE SYKES

(C)

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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IN

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Dedicated to

MUM and DAD

for their continuous support

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through the years

ABSTRACT

This work was primarily devoted to the development and evaluation of radiolabeling and radioassay methods for the chemotherapeutic drug cis-dichlorodiammineplatinum(II) (cis-platinum) and its effects on the biological behavior of the radiopharmaceutical ^{67}Ga-citrate. More sensitive and refined techniques of cis-platinum detection in biological samples would be useful in the ongoing studies of its pharmacology and therapeutic potential. The role of ^{67}Ga-citrate as a diagnostic tumor localizing agent would be substantially improved by understanding its behavior under specific iatrogenic conditions.

Radiolabeling was assessed using current synthetic methods and via a direct activation technique. The labeled compound was found to be most efficiently produced by a time limited thermal neutron bombardment of the final compound followed by chemical and radiochemical purification and subsequent solid scintillation detection of the X-ray emissions. This material was utilized for a short term biodistribution study and serum protein binding experiments. In addition, a neutron activation analysis method was developed for the detection of platinum which allowed its low level quantitation in complex biological matrices.

The ability of cis-platinum to influence the biological behavior of ^{67}Ga was investigated *in vivo* and *in vitro*. In simple solutions, cis-platinum and K_2PtCl_4 decreased ^{67}Ga binding to human transferrin under specific conditions, but the drug did not directly effect ^{67}Ga-citrate binding to human serum proteins *in vitro*. Studies in

rodents indicated the most prominent effects of drug treatment on ^{67}Ga biodistribution occurred after the development of cis-platinum toxicity, which could then influence both the short and long term tissue deposition. More extensive studies in rabbits indicated these toxicities included changes in renal function, serum iron parameters and nutrient intake. The compromised handling of the radiopharmaceutical in these animals was manifest by diminished elimination, slightly enhanced serum protein binding, altered blood kinetics and increased tissue accumulation. These observations should be of value for the correct interpretation of future ^{67}Ga-citrate scans of patients treated with this drug, and provides a mechanistic basis for understanding ^{67}Ga behavior under a variety of 'abnormal' physiological conditions.

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INTRODUCTION

Since the beginnings of nuclear medicine in the late 1940's, there has always been the cherished hope that some radioisotopic agent would be developed with a unique affinity for malignancies without regard for anatomical site, size, tissue of origin or histopathology[1]. Such an agent should be capable of non-invasive tumor detection, the assessment of local extent and distant metastasis, and also aid in the evaluation of therapeutic measures and follow-up treatment by giving some indication of tumor cell viability. This concept of the 'magic bullet' remains the ideal model in the search for useful tumor localizing radiopharmaceuticals.

Although the development of new, more useful agents is a major goal for non-invasive tumor diagnosis in nuclear medicine, another important area of investigation, perhaps with more immediate and practical significance, involves the biological handling of available agents under various conditions, with hopes of improving their diagnostic potential. This is especially important in current practice in cases where 'abnormal' tracer uptake is evident, and which may be due to a variety of factors other than the presence or absence of malignant disease. In the clinical setting, where iatrogenic intervention is routine, each change in a patient status may be important in deciding the correct interpretation of a tumor scan, and in turn, the appropriate therapeutic measures.

Probably the most widespread and diverse component of patient care involves the use of drugs, and thus, any drug-induced changes in pathology and physiology must be assessed relative to the ability of the radiotracer to localize in the appropriate tissues to the required

degree. There are numerous reports in the literature regarding suspected or proven cases of drug-induced alterations in radiotracer biodistribution[2,3] but very few studies accurately document the underlying mechanisms which are important in evaluating the interaction.

This work will focus on the antineoplastic drug cis-platinum and its effects on the biologiical behavior of the tumor localizing radiopharmaceutical ^{67}Ga-citrate. The spectra of activity of these agents overlap to a sufficient degree to expect concurrent usage of both agents for their therapeutic and diagnostic benefits, respectively. Abnormal ^{67}Ga-citrate scans have been observed in patients undergoing cis-platinum therapy[2] and thus, a systematic study was required to evaluate the magnitude and mechanism of such an effect. In order to accomplish this task, a wide variety of methodologies had to be developed and/or compared to assess their individual merits with respect to several analytical tests. This was especially true for the detection of cis-platinum in biological samples which required more complicated radioassay procedures. In addition, to study the interaction, an emphasis was placed on <u>in vivo</u> studies with an appropriate animal model, which would be expected to parallel the clinical situation.

LITERATURE SURVEY - CIS-PLATINUM

I. Introduction

In 1965, Barnett Rosenberg, a biophysicist at the University of Michigan, was investigating the possibility that an electric field could affect the growth processes of E. coli. He observed that low amperage alternating currents delivered through a nutrient medium by platinum electrodes caused the formation of filamentous bacteria up to 300 times the normal length. Further analysis revealed that some of the platinum from the electrodes dissolves in the medium under these conditions to form ammoniated derivatives[4], with the highest activity residing with the cis-dichlorodiammineplatinum(II) complex[5,6] (cis-platinum). The compound has since been found to inhibit cell division while not affecting the growth processes of several other bacterial strains[7,8].

Subsequently, cis-platinum was found to effectively inhibit the growth of several types of tumors in mice and rats[9]. In fact, the drug has produced a cytotoxic response at all levels studied to date, including viral, bacterial and mammalian cell cultures progressing to an antitumor response in humans. It is the first inorganic metallic drug to be accepted for trials by the National Cancer Institute, and there is hope that it represents a parent compound for a whole family of antineoplastic agents. To date, cis-platinum has established itself as a genuine benefit in cancer chemotherapy enabling improved treatment for a number of human malignancies.

II. Chemistry

Cis-platinum is a stable, crystalline yellow solid which

decomposes at 207°C[10]. It has a molecular weight of 300.0 atomic mass units with the platinum atom contributing 65% to this total. It is a square planar complex[11] with the bond angles and distances shown in Figure 1. The general reactivity of this complex is governed by the d^8 electronic configuration of the central platinum atom, the Pt (II) type co-ordination, and the cis geometry of the attached groups. The chloride ligands are referred to as the 'anionic leaving groups' and the ammine functions are called the 'carrier ligands'.

A. Aqueous Chemistry

The aqueous chemistry of cis-platinum is complicated by the formation of numerous hydroxo and aquated products. The relationship between each of these species[12] is shown in Figure 2. In addition, oligomerization products consisting of dimers ($[Pt(NH_3)_2OH]_2$) and trimers ($[Pt(NH_3)_2OH]_3$) can also form under appropriate conditions[13]. The equilibrium distribution is dependent upon the chloride ion concentration, the pH, the solution temperature, the initial cis-platinum concentration, and the presence of other nucleophiles which are capable of undergoing reaction with specific platinum forms[12,14]. Based on dissociation constants in the literature, at an initial cis-platinum concentration of 3×10^{-4} M at 20°C, a chloride ion concentration of 0.02 M and a pH of 7.0, the calculated relative amount of parent cis-platinum at equilibrium is about 73%[15]. Similar estimations are given in Table 1. Under the conditions listed, most of the drug exists as the parent molecule, with the next most populous species being the monoaquo product. This conversion takes place with a half-life of 1.7 hours in water at 37°C[16]. However, the relative

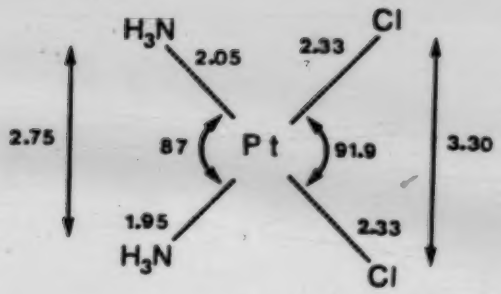


Figure 1. Structural features of the cis-dichlorodiammine platinum (II) complex. Bond angles are shown in degrees and bond lengths in Angstroms.[11]

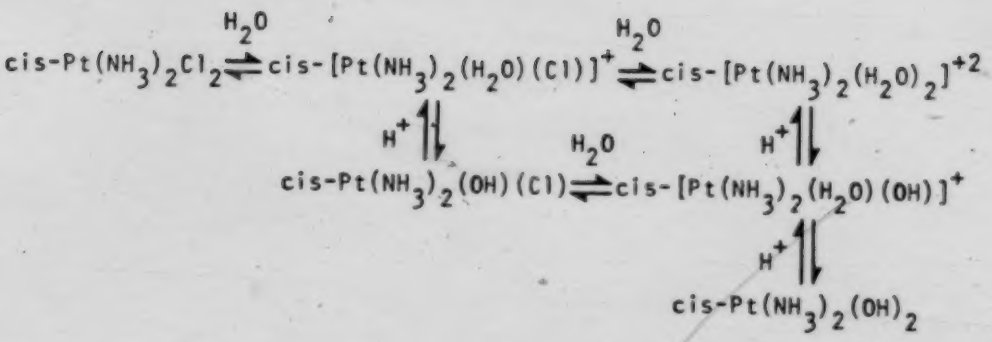


Figure 2. Aquation reactions of cis-platinum.[12]

percentage of cis-platinum drops off rapidly at lower chloride ion concentrations and depending mainly on the pH shifts to the other aquated and hydroxo species.

Table 1. Equilibrium distribution of cis-platinum (3×10^{-4} M) in aqueous media[15].

	PERCENT	
	pH = 7 $[Cl^-]$ = 0.02 M	pH = 7 $[Cl^-]$ = 0.15 M
cis-$Pt(NH_3)_2Cl_2$	72.9	96.6
cis-$[Pt(NH_3)_2Cl(H_2O)]^+$	12.0	2.1
cis-$Pt(NH_3)_2Cl(OH)$	6.0	1.1
cis-$[Pt(NH_3)_2(H_2O)_2]^{+2}$	0.2	0.0
cis-$[Pt(NH_3)_2OH(H_2O)]^+$	6.0	0.1
cis-$Pt(NH_3)_2(OH)_2$	3.0	0.1

The aqueous chemistry of cis-platinum is important in relation to its stability in parenteral vehicles and its reactivity in vivo which governs both its toxic and beneficial response. The intact molecule is definitely more stable in saline solutions and these may be necessary to deliver the drug in an form suitable for tissue penetration. The increased reactivity of the aquated forms may be responsible for the undesirable manifestations of the compound and the observed antitumor properties as well via specific interactions with biological molecules. This complex equilibrium may serve to partition the drug into specific body compartments, where a different aqueous environment determines its ultimate reactivity. The interaction with

most biological molecules is via the monoaquo and diaquo products since the reactivity of these forms is much greater than that of the other platinum species[15].

B. Structure Activity Relationships

Attempts to improve the therapeutic effectiveness and reduce the toxicities of cis-platinum have resulted in the synthesis of over 1000 analogues of the parent molecule. Only a small number of these compounds have shown antitumor effects and even fewer have proven comparable to cis-platinum[17]. However, these investigations have provided a rather detailed description of certain structural requirements necessary for active compounds of this type.

1) The compounds should be neutral. Charged complexes have been shown to possess reduced reactivity towards DNA *in vitro* and cellular penetrability is apparently compromised *in vivo*[18]. This does not necessarily mean that the active form is neutral since biological reactivity may be mediated by a charged species via ligand exchange.

2) The geometry around the central platinum atom should be square planar. This invariably necessitates the platinum to be in a (II) oxidation state[19]. These requirements control the conformation and the limits of reactivity of the complex.

3) The carrier ligand may affect the activity of the complex due to differences in basicity, electronic and steric properties[20,21]. A few compounds have been tested where these ligands were not nitrogen donors and these have shown no activity, suggesting a definite preference for ammine based systems. Platinum forms strong bonds with nitrogen ligands for thermodynamic reasons and thus these groups are

not readily exchangeable and quite inert[19]. We would not expect these substituents to be readily replaced *in vivo* but to remain part of the active platinum moiety. In general, antitumor activity of a series of nitrogen ligand donors shows the following pattern: $NH_3 < RNH_2 > R_2NH > R_3N$. Additionally, as the bulkiness of R increased biological activity is reduced[22]. These effects are difficult to relate to chemical reactivity of the platinum complex and their primary role may be related to biological properties such as hydrogen bonding stabilization, lipid membrane solubility and size related specific binding[21].

4) The leaving groups are responsible for the reactivity of the complex in light of the inert nature of the ammine ligands. However, these groups must possess intermediate leaving ability, which for many Pt(II) complexes follows the order[23,24]: $NO_3^- > H_2O > Cl^- Br^- > I^- > N_3 > SCN^- > NO_2^- > CN^-$. Labile ligands such as nitrate and water will be readily replaced by ligands present in biological molecules and thus are especially toxic. Strongly bound anions such as azide, thiocyanate, nitrite and cyanide can be tolerated in fairly high doses with no antitumor activity since very little interaction takes place *in vivo*[20]. The most active complexes contain chloride, bromide, malonate or oxalate as the anionic ligands. The latter two form bifunctional chelates and would be expected to show fairly strong binding as a result. These ligands are likely removed *in vivo* perhaps by an enzymatic process[25].

5) The leaving groups should be in the cis configuration. Thus, in a square planar complex both reactive ligands are on the same side of the molecule[26]. This is the most interesting requirement with

respect to defining the exact mode of action of these complexes.

III. Pharmacology

A. Distribution

The deposition of platinum in tissues following the administration of cis-platinum has been studied in numerous animals and in man. They all show a remarkably consistent pattern with interspecies variation likely due to changes in urinary excretion during the first 24 hours following injection. In general, the tissue uptake could be ranked as kidney > uterus > liver > skin > adrenal > ovary > spleen > pancreas > lung > muscle > testes > brain[27].

Two hours after intraperitoneal injection of cis-platinum in mice, platinum is mainly distributed to the kidneys and liver with the gut fat and spleen all showing significant uptake[28]. In tumor-bearing Swiss White mice the kidneys, liver, spleen, thymus and lungs all contain more than 1 μg of platinum per gram of tissue, 4.5 days after an 8 mg/kg intravenous dose of cis-platinum[29].

In rats, 24 hours after cis-platinum injection, the kidneys have been found to contain by far the most platinum with liver, spleen, pancreas, small intestine and lungs also containing significant concentrations[30,31]. This trend continues up to 7 days after dosing[32]. Tissue-to-blood ratios vary only slightly from 3-72 hours in major rat tissues with only kidney, liver, spleen, adrenal, bone and tumor remaining near or above blood levels during this period[33]. When tissue mass is considered, the skin, bone, muscle and liver would contain the greatest absolute amount of platinum even though their concentration values are low[27,34,35].

Tissue distribution of the drug in the female dogfish shark has also been reported. Highest concentrations were observed in the kidneys and uterus, with significant levels in the heart, rectal gland, and duodenum, followed by pancreas and spleen and comparatively little in the liver. This deposition remains relatively constant for 1 hour to 6 days after cis-platinum dosing[35,36].

In rabbits, 18 hours after drug administration, the kidneys, liver, spleen and lungs were observed to have the highest tissue uptake[28].

A consistent pattern in dogs has been observed from 10 minutes to 4 days after cis-platinum dosing with the kidneys, liver, lung, skin, ovaries, uterus and pancreas containing the highest concentrations of platinum during this period[36,37,38,39].

Scans of human patients treated with radiolabeled cis-platinum have shown the liver, spleen and intestines as the major sites of deposition, but only a small amount was observed in the kidneys[28,40]. The total skin mass has been estimated to contain about 5% of the initial platinum dose, 18 days after cis-platinum treatment[27,34].

The prolonged and stable retention of platinum in specific tissues has been well documented in several animal species. This is related to the equilibrium constants for tissue uptake and elimination since reports have indicated uptake is favoured by a factor of 3 to 6 or greater[41,42,43]. In addition, tissue distribution has been shown to be relatively unaffected by initial cis-platinum dosage in the range 0.066-3.15 mg/kg by intravenous bolus[27] or the bolus concentration[30].

The lack of specific tumor uptake indicates the drug is not

preferentially distributed to tumor cells and thus radiolabeled cis-platinum would not be of value as a diagnostic tumor scanning agent. The presence of a tumor in animal models has been shown to apparently affect the overall handling of the drug. Tissue concentrations are increased in mice[29] and decreased in rats[33] bearing tumors a few days after dosing, perhaps reflecting increased clearance[29,33] or organ weight changes[29] in these animals compared to controls.

The tissue distribution may also serve as a guide to potential therapeutic or toxic responses to the drug. There are measurable levels in the ovaries, uterus and testes and the spectrum of activity clearly cover tumors in this area. Renal damage is likely related to the vast amount of the drug presented to the kidneys. The cerebrospinal fluid levels in monkeys have been shown to be about 60 ng/ml with a half-life of about one hour[44], and similarly low levels have been found in human brain and brain tumor tissue[45,46]. These small amounts are responsible for the cytotoxic response and a possible centrally mediated emetic action. However, in all cases studied to date, the presence of platinum per se does not constitute sufficient grounds to expect any response, since it is the nature or reactivity of the deposited platinum species which will determine the cellular and subsequent tissue consequences.

B. Excretion

The excretion of platinum derived from cis-platinum administration occurs almost exclusively via the urine in all animal systems studied. However, there is considerable variation as to the

rate and extent of this process. Overall fecal elimination is comparatively small.

The urinary excretion of platinum in mice has been found to be extremely rapid initially, with almost 80% being lost in the first 24 hours[28]. Thereafter total body excretion processes are considerably slower, so that by 5 days only about 14% remains[27,28] and by 15 days approximately 10% of the dose is left in the body[29].

In rats, the largest portion of the dose has been shown to be eliminated in the urine during the first 2 hours (~ 25%)[47] and by 1 and 4 days about 30%[47] and 50%[37], respectively, have been excreted by this route. Fecal excretion in the rat accounts for less than 2% of the dose in 24 hours and more than 80% of this appears via the bile. Both urinary and fecal elimination percentages have been shown to be independent of the cis-platinum dose in the range 0.01-6.0 mg/kg in the rat[47].

Four hours after cis-platinum bolus administration to dogs, 35 - 60% of the injected platinum can be found in the urine[37,48], indicating an initial rapid renal excretion with a half-life of less than 1 hour[37]. By 48 hours about 80% has been eliminated in the urine[36,39] and thus the second elimination phase is much slower[37]. Very small amounts of platinum have been detected in the bile of dogs[37], indicating fecal excretion is low.

In contrast to these animals, the dogfish shark reportedly excretes less than 10% of the total platinum dose even after 6 days post cis-platinum administration[36].

It has been shown that humans excrete considerably less of a cis-platinum dose in the urine than the mouse, rat or dog. Total renal

elimination of platinum 24 hours after intravenous bolus dosing ranges from 20-35%[49,50] and similarly, 19-35% after completion of a 1/2-1 hour infusion[51,52]. By 5 days, 27-45% is excreted in the urine, with an estimated elimination half-life of 8-10 days[49]. A 20 hour infusion results in less urinary excretion (21-26% in 2 days[53]), while administration by a 5 day infusion has been found to greatly reduce the value to about 10% in 2 days[54]. It has been estimated that less than 10% of the dose is excreted in the feces[49], likely via the bile[45,55].

Renal handling of cis-platinum appears to be primarily by glomerular filtration[51]. Several reports have indicated both parent drug and metabolites are present in the urine[50,56]. The nature of the urinary platinum compounds appear to be mostly unchanged cis-platinum initially, in a similar proportion to the parent compound in the infusion solution. A second species (thought to be $cis[Pt(NH_3)_2(H_2O)Cl]^+$) increases with time at the expense of cis-platinum. A third species[57], possibly bound to a macromolecule[56], has also been detected. These quantitative changes with time are consistent with a hydrolytic equilibrium between platinum species in the urine which are related to urinary chloride ion concentration[57]. These processes would explain the observed changes in urinary elimination observed after infusion dosing protocols.

C. Plasma Protein Binding and Pharmacokinetics

After intravenous injections in humans, cis-platinum has been found to associate almost exclusively in the plasma component of blood. The interaction with plasma proteins is considerable, with

about 90% binding after several hours[27]. In vitro studies have indicated the cis-platinum serum protein interaction takes place with a half-life of 156-220 minutes[13,51,58]. Serum from cis-platinum treated patients shows no dependence on chloride ion concentration for platinum release[17] but does affect the association in vitro[59]. No dissociation of platinum has been observed from protein bound solutions even after dialysis for 24 hours[56,60]. These facts support the position that the reaction with serum proteins is one of covalent bond formation rather than involving a hydrogen bond, electrostatic or van der Waals forces. Almost all plasma proteins appear to be involved in the process including gamma globulins, albumin and transferrin[53,60,61]. This plasma protein interaction is highly significant since the protein bound cis-platinum has no cytotoxic activity[56,62]. In addition, the pattern of binding appears to be at best only slowly reversible, meaning that therapeutically active drug is being removed from the system by an almost irreversible process.

In humans, a small portion of the intravascular drug has been found in the red blood cells (RBC)[31,37], the majority being taken up immediately after injection, when total blood concentrations would be greatest[63]. The RBC associated platinum is intracellular, although a small portion may be membrane bound[61,63,64]. The RBC platinum levels remain relatively constant with time, while the plasma levels are declining. However, only about 1% of the total blood platinum is normally found with the RBC fraction. In rats, the reported association of cis-platinum with RBC was considerable, since by 1 hour more than 60% of the blood platinum was RBC associated, the vast majority of which was intracellular[61]. The interaction of platinum

with leukocytes has been reported as 6-11% of the coincident plasma concentrations[50]. Thus, the overall distribution of cis-platinum in the cellular blood component is rather limited and likely insignificant in terms of the therapeutic potential of the drug in man.

The pharmacokinetic parameters related to the plasma disappearance of platinum species following intravenous administration have been summarized in Table 2.

The human data suggests an initial half-life ($t_{1/2}\ \alpha$) of about 30 minutes and thus by 2 hours less than 7% of the dose remains in the circulation. In fact, a faster phase may also be present which would further increase blood clearance[27]. The longer half-life ($t_{1/2}\ \beta$) is of the order of several days and is likely a consequence of the extensive protein binding but represents only a small portion of the dose. The filterable platinum kinetics reported refers to plasma platinum which is free or associated with plasma components of molecular weight less than 10-25 thousand. These kinetics are the same for the initial phase, indicating this phase represents clearance of free cis-platinum. The absence of a $t_{1/2}\ \beta$ is indicative of the rate of plasma protein binding[65,66].

Table 2. Summary of reported pharmacokinetic parameters of cis-platinum.

Species	Mode of Administration	$t_{1/2}\ \alpha$ (minutes)	$t_{1/2}\ \beta$ (days)	Ref	Comments
rat	IV bolus	43	2	36	
rat	"	8.6	44.9	34	
rat	"	10	1.49	32	
rat	"	16.1	6.6	34	
rat	I.P.	-	2.5	32	
shark	IV bolus	69	10	36	
shark	"	71	17.5	35	
dog	"	22	5	36	
dog	"	<60	4-5	37	
dog	"	12	2.3	43	
dog	"	30	-	39	filterable Pt*
dog	"	143	-	38	filterable Pt*
human	IV bolus+	25-49	2.4-3.0	50	
human	"	-	3-5	49	
human	"	7	1.1	34	
human	"	32-54	-	57	filterable Pt*
human	"	34	4.5	55	
human	"	21.3	6.2	41	
human	1 hr infusion	23	2.8	56	
human	6 hr infusion	17.5-37.1	-	57	filterable Pt*
human	6 hr infusion	-	3.0	55	
human		78	-	43	filterable Pt*
human		25	1.8	42	
human		25-30	1.8	43	
human	5 day infusion	-	1.4	54	

+ administered over < 15 min period; * non-protein bound or free platinum (see text)

IV. Biochemistry

A. Intracellular Localization

Cis-platinum has been shown to bind to the erythrocyte cell membrane, possibly with the amino acid residues of the protein component[64]. Interactions at the lymphocyte cell membrane apparently cause unmasking of lectin binding sites by removal of mucopolysaccarides from the cell surface. Exposure to the drug for longer than 60 minutes destroys these binding sites on lymphocyte, lymphoma and ascites cell membranes[67].

Administration of cis-platinum[68,69] or cis-platinum-DNA[70] or -uracil[69,71] complexes have been shown to result in platinum localization in lysosomes or specific vacuoles of the analyzed cells, and have been termed platinosomes[70,71]. The significance of this deposition has not been determined. Ultramicroscopic studies of cultured cells have shown the presence of platinum along the entire inner surface of the nuclear membrane, in the nucleolus and other localized patches in the nucleoplasm[72].

Quantitatively, the cytosol contains the most platinum after *in vivo* administration of cis-platinum (at least for kidney and liver cells)[32,73] and much of this appears to be protein bound[74]. The other major compartmentalization occurred in the microsomal[32,74] and nuclear[73,74] fractions. These studies demonstrate that a variety of potential intracellular targets exist for the drug and that the mechanism for the eventual cytotoxic or other consequences cannot be considered a function of a single site of localization.

B. Interaction with Enzymes

Various platinum halide complexes have been known for many years to bind to enzymes[75]. In vitro inhibition has been demonstrated in numerous systems. Several factors regarding the nature of the complex may be implicated in this effect including the rate of hydration and aquation[76,77], electrostatic charge[78], the number of labile ligands[9] and geometric size[79]. The presence of competing ligands[80,81,82] and the structure of the enzyme itself[83] are also involved. Cis-platinum has been shown to depress the activity of several specific enzymes: lactate dehydrogenase, alcohol dehydrogenase[84], malate dehydrogenase[78,79,84], NAD hydrogenase, glyceraldehyde-3-phosphate dehydrogenase, glucose-6-phosphate dehydrogenase and dihydrofolate dehydrogenase[84] are all inhibited by the drug in aqueous solution. This effect is likely due to platinum binding at essential sulphydryl groups, since enzymes which do not possess this moiety are generally not inhibited[83]. In kidney extracts, testosterone dependent esterases[85] and Na^{+}/K^{+} or Mg^{+2} activated ATPases[82] are both inhibited. In vivo, significant reduction in enzyme activity has been demonstrated for dihydrofolate reductase, thymidine kinase and thymidylate synthetase[86,87]. The drug does not effect the performance of enzymes involved in DNA[88,89,90] or RNA[91] replication.

C. Interaction with Nucleic Acids

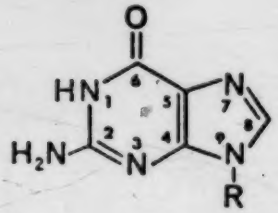
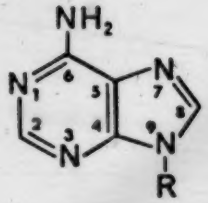
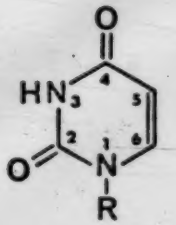
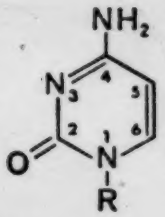
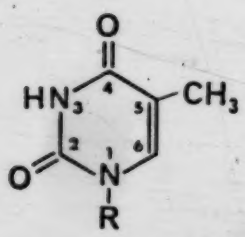
The interaction of cis-platinum with individual nucleic acids has been studied extensively in vitro. Analysis of the reaction products under various conditions has been accomplished using sensitive instrumental physico-chemical techniques[92]. The location of

platinum binding sites on the bases can be determined by blocking available sites by methylation or protonation. The most important interaction, in terms of its relevance to the *in vivo* situation, will be with nucleotides, since their binding sites most resemble those potentially available in the DNA molecule. A summary of the cis-platinum reactions with the individual nucleotides is shown in Table 3. A variety of binding sites have been detected, but for guanosine and adenosine the N(7) site is the primary point of attachment as is the N(3) position of cytosine[93,94,95,96,97]. Thymidine and uridine do not react via N(3) under normal conditions possibly due to their lower basicity or steric effects[93]. Since the lone pair electrons in the amino groups of the purine rings participate in the system, this group is generally not involved in metal binding[98].

Cis-platinum also reacts with specific dinucleotides. Various adenosine-cytosine dimers bind via the C(4)-NH_2 position of cytosine and the N(1) position of adenosine only when the bases are in a stacked configuration such that the geometrical conditions for cis-bidentate binding are met[99]. Guanine dimers[100] and G-C-G trimers[101] both form N(7) to N(7) chelates in the presence of cis-platinum. Similarly, artificial or natural polynucleotides containing guanine form a platinum intrastrand crosslink between this base's N(7) binding sites[102].

A novel interaction between cis-platinum and the slow reacting thymidine and uridine nucleotides involves the formation of the 'platinum blues'. Characteristic properties include containing a mixed valency platinum species with oligomeric structure and

Table 3. Location of cis-platinum binding sites on nucleosides *in vitro*[93-97].

		Monodentate	Bidentate
Guanosine		N(7)	C(6)-O, N(7) (?)
Adenosine		N(7)	$C(6)-NH_2$, N(7) $C(6)-NH_2$, N(1)
Cytosine		N(3)	$C(4)-NH_2$ N(3)(?) C(2)-O, N(3)(?)
Thymine		-	-
Uridine		-	-

(?) Indicates this mode has been disputed and may be sensitive to reaction conditions and analytical techniques

paramagnetic behavior[103,104]. These complexes are likely localized Pt(III) and Pt(II) atoms, which as a chain of amidate bridged platinum atoms display a formal non-integral oxidation state[105]. The actual reaction is dependent upon several factors and only a small percentage of the products generate the blue colour[106]. This group of compounds have been studied for their therapeutic potential[107,108] and as a mechanism for the cis-platinum antitumor effect via the formation of stacked polymers of platinum interacting along the DNA chain[103].

Cis-platinum has been shown to form a weak complex with pyrophosphate[109] and be generally unreactive in phosphate buffers[110] where polymers or aggregates apparently form[111]. Specific monophosphorylated[112] and triphosphorylated[110] nucleic acid derivatives have also been reported to have phosphate participation in their in vitro reaction with cis-platinum. However, studies with DNA seem to exclude this type of binding[94], unless, as in the case for the trans isomer, it is of a hydrogen bonding nature[113]. No association of cis-platinum with the cyclic ribose or deoxyribose sugars has been observed, although the drug does react with the straight chain sugar D-mannitol[114].

V. Mechanism of Action

A. Inhibition of DNA Synthesis

The biochemical reactivity in vitro and the biological activity in vivo both suggest that the primary target of the active platinum species is DNA. Its ability to inactivate viruses[111,115], cause induction of lysogenic bacteria[116], produce filamentous bacteria[6], prevent mitosis[117] and induce mutagenesis[118,119] are all consistent

with an effect on DNA. More importantly, it has been shown in human amnion AV_3 cells[120], Ehrlich ascites tumor cells[121] and phytohemagglutinin stimulated lymphocytes[122] that low doses of cis-platinum produce profound effects on DNA synthesis, even in cases of high cellular viability, while RNA and protein synthesis are only slightly depressed. This effect is not due to inhibition of DNA polymerase activity[88,89,90], nor DNA associated protein binding[123], but rather a direct action on the template itself. Only specific platinum complexes with the cis configuration are capable of eliciting this response. Both trans- and cis-platinum are associated with intracellular DNA in a similar quantitative manner[15,124] indicating the same degree of cellular penetration and reactivity. However, the resultant cytotoxicity and antitumor properties of the cis-isomer are due to its ability to produce a highly specific DNA lesion which impairs cellular reproduction and eventually causes cell death. The preferential binding site in DNA is attachment to guanine residues. There is a direct correlation between the degree of platinum bound and the G-C content of the DNA[125,126]. In enzyme cleaved DNA, pre-bound cis-platinum is found only in G-C rich regions[127]. However, the exact nature of the lethal lesions has not been identified. Two modes of binding are thought to be involved: an intrastrand reaction involving either a bidentate chelation to the N(7) and C(6)-O position of guanine or a N(7) to N(7) crosslink of adjacent guanine bases; or the interstrand crosslinking of guanine to other residues on the complementary strand.

Interstrand crosslinks have been observed in many studies of cis-platinum-DNA interactions[120,124,128] and in several cell systems

the degree of interstrand crosslinks correlated with the cytotoxic effect[129,130,131,132,133], although not all[133,134]. However, the trans isomer appears to be equally effective in the formation of this type of crosslink and is ineffective as an antitumor agent[135]. It has also been reported that only a small percentage (0.1-3.3%) of DNA bound platinum is involved in interstrand crosslinks[111,136,137,138,139] and thereby it is suggested that this interaction probably contributes little to the overall inactivation process[111,126].

Indirect evidence for intrastrand crosslinking of DNA by cis-platinum has been obtained from studies on mutagenicity[140], interference of interchelator binding[141,142,143] and observed structural changes[136,144,145,146]. More direct evidence is available from studies of polynucleotide[147] and DNA[126,136,148] binding with cis-platinum. This mode of binding is favoured in light of the strict stereochemical requirements, found only in the cis-isomer, necessary for antitumor activity.

The intrastrand crosslink would likely take two forms. The first involves bidentate chelation to a single guanine base via N(7) and C(6)-O. This mode has been proposed[98,136] and supported by isolated DNA studies[94,149], although detection methods have been questioned[92]. It has not been verified by X-ray crystal[150] or UV analysis[151] of cis-platinum-guanine complexes. Enzymatic systems in human cell lines which are capable of repairing C(6)-O guanine adducts (such as nitrosoureas) do not alter cis-platinum cytotoxicity[131]. It may be possible that a hydrogen bond interaction may occur with the N(7) guanine bound cis-platinum molecule between one of its ammine ligands or a coordinated water molecule[97,152] and the C(6)

oxygen[98,153] or a phosphate oxygen[154]. This is especially attractive in light of the structure activity relationships of the ammine group associated with the central platinum atom[25]. The proposal that the C(6)-O and N(7) chelate causes the release of the N(1) hydrogen and subsequent mispairing of the bases has not been supported by molecular orbital calculations[155,156] but is suggested by electrophilic considerations[95].

The coupling of adjacent guanine bases is the second binding mode favoured for the formation of a intrastrand crosslink. Cis-platinum has a high affinity for the N(7) guanaine site from both a thermodynamic[157,158] and kinetic viewpoint[110]. For this reaction to occur, local pertubation in the helical DNA structure must take place and have been observed experimentally[136,144,145,146,147]. Reactions with various poly G-C nucleotides have shown this mode to occur to a greater extent when the guanine bases are in the stacked configuration as expected[102]. Although the requirement for a large number of adjacent guanine residues seems prohibitive[147,142] evidence for intrastrand crosslinks between guanines in G-G[159], GAG or GCG segments of DNA[160] and trinucleotides[101] has also been reported. Crosslinking between guanine and adenine on the same strand would be in disagreement with the recognized guanine-cytosine specificity, and guanine to cytosine linkages have been intimated[161] but not verified by <u>in vitro</u> studies[162].

Alternate binding modes, which by themselves are inconsequential, may be involved in determining the lethal lesion by generating or inhibiting a specific platinum binding site.

The specific cis-platinum lesion could be considered more

resistant to biological repair systems than those of trans-platinum. Thus, different cell repair capabilities may account for the preferential toxicity and the mechanism of antitumor activity. Early studies in bacterial and mammalian cell cultures suggested that cells are capable of repairing damage induced by cis-platinum. In particular, cells with excision deficient processes were much more sensitive to the drug compared with normal cells[124,163,164,165,166]. The effects were related to the time and dose-dependent excision repair of cis-platinum crosslinks in DNA[167,168,169]. However, since the removal of interstrand crosslinks may parallel removal of other platinum adducts, one cannot specify which lesion is responsible for the cytotoxicity[170]. Excision repair *in vivo* has also been used to account for the preferential tumor cytotoxicity relative to normal cells[171].

Single strand gaps or breaks have been observed in newly synthesised DNA of cis-platinum treated cells[172], especially in the presence of caffeine[173,174]. Caffeine is a known inhibitor of the second type of repair process known as postreplication repair[174] and thus it would appear both systems play a role in determining the cytotoxicity of this drug.

B. Other Proposed Mechanisms of Action

The diversity of biological actions attributed cis-platinum have caused several investigators to look elsewhere for effects which may also be involved in its cytotoxic response. These alternate mechanisms have been proposed and experimentally supported despite the well recognized effects on DNA synthesis.

Although the major effect of cis-platinum treatment is a reduction of DNA synthesis, it is apparent that the DNA damage can be repaired and growth continued. It may be possible that metabolic consequences are responsible for the cytotoxicity of this compound. In vivo inhibition of respiratory processes in P-388 leukemia cells by malonato-diammine-platinum (II) have been observed[87]. Oxygen consumption in Hela cells is reduced by cis-platinum[175] as is respiration in isolated mitochondria at high drug concentrations[176]. These effects may be independent from the DNA action and contribute to the eventual cell death.

Another second proposed mechanism relates to the ability of the drug to induce mutagenesis, an effect observed in all bacterial and mammalian systems studied to date. It produces both a base-pair substitution[119,177-183] and frame-shift[184,185] mutations in several bacterial strains. In hamster embryo cell cultures chromosome abberations such as sister chromatid exchange have been demonstrated[186]. Human or rabbit lymphocyte cultures exposed to cis-platinum at the G phase of the cell cycle also show sister chromatid exchange[187], but less so than those exposed during the S phase[188]. Administration of the drug to rats or rabbits produces lesions in circulating lymphocytes, bone marrow and thymic nodes cells that are expressed as chromosome abberations upon culturing[118,187]. Thus, the intracellular consequences of mutant formation may ultimately lead to cell death.

The immunologic mechanism for the antitumor action of cis-platinum is debatable in light of numerous contradictory reports. It has been found to decrease the antibody-plaque forming ability of

mouse spleen cells[189,190] which lasts three days after a single dose[189]. Cultured human lymphocytes, stimulated with phytohemagglutinin, showed a depressed immune response after cis-platinum treatment[122]. Lymphocyte blastogenesis was significantly reduced in patients receiving the drug[191]. Cis-platinum also prolongs the ability of treated mice to retain skin grafts[192] and affects their allograft reaction[193]. At non-toxic concentrations, cis-platinum was found to depress the chemotactic response of macrophages in vitro[194]. These results tend to support the immunosuppressive action of the drug.

In contrast, the immune competence of thymic-dependent cells from mice was not affected by treatment[195]. In vivo, macrophages are observed in cis-platinum treated tumors but are absent in control tumors[196,197]. The co-administration of immunosuppressive drugs reduced the observed cure rate, while immunostimulants enhances its effectiveness[198]. It has also been found to slightly increase spontaneous cell-mediated cytotoxicity of lymphocytes[199] and monocytes[200]. This apparently enhanced antigenicity of tumor cells is not related to a specific membrane interaction since both normal and malignant cells show membrane associated platinum[201]. These latter studies suggest an immunostimulant effect of cis-platinum may contribute to the observed antitumor response.

VI. Clinical Applications

A. Chemotherapy of Cancer

Cis-platinum has been evaluated for antineoplastic activity in a large number of clinical studies. These phase I-III trials have

established a fairly wide spectrum of neoplasia where the drug has definite utility. A brief summary of the most recent reports dealing with specific clinical applications of the drug for human cancer chemotherapy will be presented.

Cis-platinum is currently included in almost all standard chemotherapeutic regimens for the treatment of ovarian cancer. The drug possesses significant activity as a single agent[202] and in particular with doxorubicin[203]. This combination has been found more effective than alkylating agent therapy including thiotepa or with methotrexate[204], and is effective even in certain refractory cases[205] with no apparent histologic specificity[202]. Overall response rates of 40-60% have been reported for various cis-platinum combinations in the treatment of ovarian cancer[202-205].

The single agent overall response rate to cis-platinum for the treatment of squamous cell carcinoma of the head and neck has been reported as 30%[206], and in combination with methotrexate, bleomycin or 5-fluorouracil this figure is increased to about 80%[207,208]. The use of pre-operative cis-platinum combination therapy produced significant improvement in subsequent surgery, irrespective of histologic classification[209].

Cis-platinum treatment of testicular cancer has been a major development in the field of antineoplastic chemotherapy. The drug has proven highly effective in the treatment of both seminomatous[210] and non-seminomatous[211] tumors of testicular origin. The most common cis-platinum regimen includes vinblastine, bleomycin and doxorubicin occasionally with cyclophosphamide[211]. The cure rate with such treatments can approach 100%, a figure that is virtually unparalleled.

Conventional chemotherapeutic agents have been of little or no benefit in the treatment of esophageal cancer, with overall responses of less than 15%[212]. The use of cis-platinum in single agent therapy has produced a 30% response rate[213] and in combinations with bleomycin, vindesine[212] and methotrexate[214] this has been improved to 55%. Further benefits may be realized since pre-operative treatment has resulted in a large percentage of patients presenting resectable tumors[212].

The efficacy of cis-platinum alone in the treatment of squamous cell carcinoma of the cervix ranges from 20-50%; with the lower response rate observed in patients who have received prior chemotherapy[215]. In many cases, the drug apparently stabilizes the tumor to prevent further growth[216]. In combination with mitomycin-C, bleomycin and vincristine for the treatment of either advanced or recurrent disease, a 43% overall response rate has been achieved with most (29%) being complete responses[217].

Cis-platinum drug combinations have been reported to be the most effective chemotherapy for advanced bladder cancer[218] with an overall response rate of 45%[219]. The combination with doxorubicin and cyclophosphamide appears to be especially promising[218] (82% response) in comparison to standard therapy using doxorubicin, mitomycin-C and 5-fluorouracil (27-33% response)[220].

The exact role of cis-platinum in various lung cancers has not been fully established. It is of limited value when used alone even at high doses[221]. In combination with cyclophosphamide and doxorubicin variable responses from 7%[222] to 42%[223] have been reported. The inclusion of vindesine in this regimen produced a high

range response[224] and in combinations with other agents showed parallel success[225]. Thus, it appears cis-platinum combinations can be effective in the treatment of lung cancers but the precise factors for maximal response have not been defined.

Initial clinical evaluation of cis-platinum in several other tumor classes has also been performed in a limited number of patients. The observed potential from these preliminary studies indicates the therapeutic utilization of cis-platinum will undoubtably be expanding in the near future.

B. Radiation Sensitization

In 1971, it was observed that mice treated with cis-platinum after irradiation greatly enhanced the observed lethality[226]. This combination was also shown to increase the lifespan of lymphocytic leukemia diseased mice by a factor greater than either treatment alone[227]. *In vitro* studies using *Baccilus megaterium* spores[228] and *E. coli*[229,230] indicated that cis-platinum was capable of enhancing the radiation induced lethality in the presence or absence of oxygen. Studies with murine mammary adenocarcinoma[231] and lymphoma[232] have indicated that cis-platinum and therapeutic radiation effects are only additive. However, in Chinese hamster ovary[233], lung fibroblasts[234] and intracerebral rat tumors[231], this effect is synergistic. It appears to be drug dose-related and cell cycle dependent[235]. Cis-platinum has also been investigated in combination with 6 MeV neutron therapy and found to be supradditive[236]. The ability of cis-platinum to act as a hypoxic cell radiosensitizer may be related to hydroxyl radical yield enhancement, charge sequestration, or

neutral radical oxidation processes[237]. It may make the DNA more susceptible to radiation damage but most evidence supports the idea that it acts by inhibition of radiation induced sublethal or potentially lethal damage repair mechanisms[236,238]. The synergistic effects of cis-platinum and radiation has also been observed in normal tissue[238] and thus considerations of reduced drug or radiation dose should be considered in patients receiving both therapeutic modalities.

VII. Administration and Dosage

In view of the aqueous chemistry of cis-platinum, it is not surprising to find the drug is readily transformed into aquo and hydroxo byproducts upon dissolution in water, but is stabilized in chloride containing solutions. The intact molecule predominates (>96%) in normal saline solutions even after 24 hours[14]. Precipitation of cis-platinum from refrigerated solutions occurs if the concentration is greater than 0.6 mg/ml[239], or if the solution contains sufficient base[14]. Exposure to intense fluorescent lighting[239] and elevated temperatures[14] should be avoided in the storage of cis-platinum admixtures. The addition of mannitol or dextrose to saline vehicles containing cis-platinum produced no adverse effects. Variation of the saline or related salt solutions of cis-platinum for vehicles has been studied in mice. When the drug was given in hypertonic (4.5%) saline, a large decrease in lethal toxicity was observed compared to normal saline and related to a reduced tissue uptake, faster plasma clearance with less plasma protein binding. The antitumor properties were apparently unaffected[240]. By shifting the equilibrium towards the

parent dichloro molecule, the more reactive species are initially suppressed and the corresponding toxic response reduced. This approach has not been attempted clinically and the preferred vehicle for cis-platinum administration remains either normal saline or saline with 5% dextrose.

Adequate cis-platinum dosing requires the assessment of several factors, including dose fractionation, infusion protocols, route of administration and the use of therapeutic adjuvants. During initial clinical trials, the drug was given in various doses and dosage schedules, and these have evolved into three common dosage regimens.

(I) 100 mg/m^2 once monthly

(II) 20 mg/m^2 daily for five days repeated once monthly

(III) 30 mg/m^2 once weekly

Each course results in the delivery of about the same quantity of drug but vary in terms of fractionation. The impact of daily (II) or even weekly (III) treatment on patient compliance with respect to the induction of nausea, vomiting and anorexic side effects would favour the use of schedule (I)[43]. The recovery of DNA synthesis in normal tissues after cis-platinum treatment is apparently faster (2-3 days) than in tumor cells (6-8 days)[27]. Thus, the once weekly treatment (III) would maximize the relative recovery periods between normal and malignant cells. Both therapeutic and certain toxic effects may be related to peak plasma levels. Intra-arterial injections are generally superior to intravenous administration in locally confined tumors[241], while nausea, vomiting[54,242] and ototoxicity[243] are more severe in patients treated by intravenous bolus injection compared to prolonged infusions. These properties may

be more a reflection of free versus plasma protein bound cis-platinum since similar toxicities have been observed with a regimen similar to I and given by the same infusion protocol[43]. Renal and hematopoietic effects may be less influenced by peak plasma levels since these toxic responses have occurred similarly in dogs treated either by intravenous or intraperitoneal injections[48,244].

In vitro the drug achieves roughly the same degree of cell kill during short exposures to high concentrations as with longer exposures to low concentrations[245,246], suggesting a relationship based on the product of concentration and time. In leukemic mice, there is no apparent advantage of prolonged infusions over single bolus injection. Higher doses can be tolerated by infusion, but are necessary to achieve similar survival rates and toxicity[247]. Therefore, it would seem that any benefit in efficacy due to prolonged infusions would likely be realized as a decreased toxicity rather than improved antitumor effect.

The use of patient hydration in combination with mannitol and/or furosemide is necessary to prevent dose limiting renal toxicity. The induction of brisk diuresis with adequate fluid replacement[248,249,250] has been shown to reduce intratubular cis-platinum concentrations during the period of increased renal flow without interfering with the therapeutic properties[47]. An out-patient protocol has been developed involving these principles and results in a low incidence of predominantly mild renal abnormalties[251].

Cis-platinum has been encapsulated in several types of vesicles commonly termed liposomes. Administration of the drug in this form generally results in increased tumor uptake [252,253] and delayed plasma

clearance[252,253,254]. There is no increase in renal toxicity such that the overall therapeutic benefits should be increased[253,254]. The actual antitumor response of this form of cis-platinum usage has not been measured.

The use of intratumoral injections in mice of water in oil emulsions of the drug have proven to reduce the toxicity while maintaining high therapeutic activity, in comparison to the intravenous route[255].

VIII. Toxicity

A. Animal Toxicity

The dose-related morbidity and lethality of cis-platinum in various animals is summarized in Table 4. Animals receiving LD_{50} doses either die in 3-4 days of acute toxicity or survive and recover in 2-18 weeks[256,257]. The major pathological changes which occur in mice and rats include: sloughing and denudation of the gastrointestinal mucosa (mainly the small intestine[258]); pycnosis and vacuolization of the renal tubular epithelium leading to acute nephrosis; leukopenia due to bone marrow suppression and atrophy of the spleen and thymus[256,259]. Toxic manifestations in the dog and monkey have been shown to be quite similar. Emesis, anorexia, diarrhea and abdominal tenderness were apparent signs of gastrointestinal damage such as hemorrhagic enterocolitis. Hematopoietic lesions such as hypoplasia of the bone marrows and lymphoid tissue atrophy were also reported. The most severe toxicity affects the kidneys with dogs showing renal tubular necrosis and monkeys displaying renal nephrosis[256,259]. A large amount of data obtained from animal studies

will be presented with the appropriate clinical toxicity discussions.

Table 4. Cis-platinum animal toxicity.[257]

Animal	LD_{50} (mg/kg)
mice	12-14
rats	11-13
young chickens	5-6

Table 5. Cis-platinum toxicity data in beagle dog and rhesus monkey.[256]

	Dog		Monkey
	(mg/kg)		(mg/kg)
	single IV dose	daily IV dose x 5 d	daily IV dose x 5 d
HNTD	0.625	0.187	0.156
LTD	1.25	0.375	0.313
HTD	2.5	0.75	1.25
LD	5.0	1.5	2.5

HNTD = high non toxic dose LTD = low toxic dose

HTD = high toxic dose LD = lethal dose

B. Clinical Toxicity

1. Nausea and Vomiting

This side effect has been observed in all patients within a few hours after single intravenous doses of 0.75 mg/kg and in a large percentage of patients receiving 100 mg/m^2 as a 6 hour infusion[260]. Virtually every clinical report indicates this effect of the drug is extremely common and often very troublesome in terms of patient compliance. Various methods to ameliorate this problem have been attempted.

The synthetic narcotic antagonists such as Nabilone produced poor results in dogs[261] and humans[262] but excellent response in cats although only at doses sufficient to cause substantial behavioral side effects[263]. Common neuroleptic drugs such as droperidol and haloperidol[264,265], phenothiazine agents (chlorpromazine, prochlorperazine and thioridazine[260,261]) and diazepam[266] have shown variable and limited benefits in patients. The administration of metoclopramide is highly effective in eliminating the emetic side effects of cis-platinum in ferrets[267], dogs[261] and man[260,268,269,270]. Steroid therapy using dexamethasone[271,272] and methylprednisolone[273] have also been shown to be quite successful in inhibiting cis-platinum induced nausea and vomiting. This is apparently not related to their inhibition of prostaglandin synthesis since prostaglandin levels are not elevated during the episodes[274]. Slow infusions of the drug have also been found to minimize this problem[54].

The exact mechanism of this action is not known. The drug is distributed in various sections of the gastrointestinal tract and has been detected in the vomitus[275], but very low levels are found in the

central nervous system[44,45,46]. These facts, plus the reported poor efficacy of centrally acting antiemetic drugs seems to indicate a peripheral mechanism is involved. Since certain platinum compounds have been shown to release histamine in vitro[276], a local action is possible.

2. Neurologic

A small number of patients receiving cis-platinum have developed parethesia involving a decrease in vibratory, position and light touch sensations due to an absence of action potentials in nerves of the upper and lower extremeties[277,278]. Nerve biopsies have shown intermittent destruction of the myelin sheaths with no axonal involvement[279]. Less frequent motor nerve abnormalities, such as gait disturbances, are associated with decreased conduction velocities in several peripheral motor nerves[280]. Thus, the cis-platinum induced neuropathy appears to be a direct action of the drug on specific nerves, causing a degree of demyelination and impaired conduction.

3. Otologic

The incidence of cis-platinum induced ototoxicity varies with the age of the patient and the cumulative dose received. Pediatric and elderly patients appear to be especially susceptible. After a single therapeutic dose 10-30%[50,281] of patients experience some decline in hearing function while after a cumulative dose of greater than 200 mg about 90% are affected[282]. Symptoms such as tinnitus, otalgia and recruitment may be present[243], but audiometric abnormalities are more prevalent[283,284]. The hearing loss is greatest

at 4-8 KHz but may progress to the speech tones in some cases[283]. Air conduction thresholds are frequently reduced[282]. The damage is apparently progressive, and long lasting, if not permanent[284].

The mechanism for this effect is a direct action on the hearing apparatus. In guinea pigs and monkeys, the drug causes general destruction of the outer hair cells, most severely in the basal turn of the cochlea[285,286,287,288]. Specific damage includes parietal membrane dilation, softening of the cuticular plate and cellular degeneration. These structural defects result in functional impairment[289]. The effects are similar to those reported for other ototoxic drugs and have been explained by a disruption of RNA and protein synthesis, leading to ineffective membrane synthesis and inability to maintain the osmotic gradient necessary for cell function[288]. Cis-patinum secondarily affects RNA and protein synthesis at high concentrations[120]. The drug has no apparent effect on the afferent and efferent nerve fibre branches of the VIII cranial nerve[288]. The degree of ototoxicity seems to be related to peak plasma levels of the drug, since the highest incidence occurs in patients treated by intravenous bolus[283]. This is likely related to a plasma-endolymph diffusion gradient for cis-platinum penetration.

4. Hematologic

Cis-platinum treatment has been shown to reduce the concentration of several blood elements. A reduction of white cell numbers occurs after almost every therapeutic dosage or administration schedule[281]. The incidence of leukopenia depends on the clinical definition and is often difficult to quantitate because of other forms

of concommittant therapy. It seems to be dose related and occur more frequently when the drug is given at weekly intervals. The onset and nadir of leukopenia ranges from days to weeks but levels usually return to normal after several weeks[290,291]. Mild thrombocytopenia may also occur with a similar onset, nadir and recovery[281,290]. Cis-platinum induced anemia, with a positive or negative Coombs test[292,293], and a decreased hematocrit[294,295] have been reported in numerous patients, with an overall incidence from 9-40%[281].

Cis-platinum has been shown to be myelosuppressive in vivo[296] and in vitro[297], thus decreasing the stem cell population in active marrow. Circulating leukocytes are also affected and there is a direct cytotoxic action of the drug on these cells[191]. These properties would account for the leukopenia but additional factors may be involved for the anemic effect. The reticulocyte count in these patients reportedly remained constant and the observed anemia greater than if complete arrest of erythroporesis taken place[295]. This suggest a direct effect of the drug on the red cells. However, reticulocyte levels in rats are greatly reduced by treatment[299] and no direct red cell anomalies have been observed in vitro[295].

5. Nephrotoxicity

a) Diagnosis: The assessment of renal damage induced by cis-platinum treatment in man is generally accomplished by standard techniques. These consist of measurement of blood urea nitrogen (BUN), serum creatinine (SCr), creatinine clearance (CrCl), urinary proteins and enzymes. The CrCl results, being a direct measure of the glomerular filtration rate are usually more accurate, since BUN and

SCr may return to near normal after therapy despite depressed CrCl[300]. Several urinary enzymes have been used as markers of cis-platinum nephrotoxicity. Both N-acetylglucosamine and leucine aminopeptidase are associated with the proximal tubules and have been detected in elevated concentrations in the urine of treated patients, indicating kidney damage in that area[301]. β_2-microglobulin is a low molecular weight peptide normally reabsorbed by the renal tubular cells. β-glucuronidase is excluded from glomerular filtration by virtue of its high molecular weight. Thus, the reported increases in β_2-microglobulin[301] and β-glucuronidase[302] in the urine of cis-platinum treated patients can serve as a diagnostic indices of tubular reabsorption and glomerular filtration abnormalities. The time course of cis-platinum induced nephrotoxicity in animals indicates that changes in urinary parameters are capable of detecting renal impairment much earlier compared with the use of hemodynamic changes and are thus more sensitive methods in determining the extent of renal damage[303]. A radiodiagnostic method, involving the clearance of ^{99m}Tc-DTPA or ^{125}I-orthoiodohippurate has also been reported to detect acute nephrotoxic effects of the drug[304].

b) Pathology: The administration of a single intraperitoneal injection of cis-platinum to mice or rats results in progressive reproduceable structural alterations to the kidneys. Early changes (2-3 days) are indicative of the later consequences, consisting of mitochondrial changes and the loss of the brush border in the proximal tubules[259,305]. Two weeks post injection, the majority of tubules are dilated, generally devoid of microvilli, with a thickened basal lamina. These lesions become more advanced by four weeks and began to

involve the S_2 and S_3 segments of the proximal convoluted tubules. The interstitium of the outer stripe showed a marked increase in fibrotic tissue accumulation. At six months, large cysts were apparent along with collapsed atrophic tubules. This chronic damage was also reflected by cortical involvement, lymphocyte infiltration, and further interstitial fibrosis, suggesting permanent damage. Intracellular conseqences in the renal cells are mainly the presence of large atypical nuclei, large lysosomes, abundant free cytoplasmic lysosomes and rough endoplasmic reticulum[306]. Thus, in animals, the major pathologic changes appear in the S_3 (pars recta) region of the proximal tubule[306,307,308] with little or no glomerular involvement.

In humans, necrosis of the tubular epithelium, dilation of the distal convoluted tubules, moderate congestion and interstitial edema of the vasa recta, and the presence of hyaline and granular casts in the collecting ducts have been observed in the kidneys of deceased patients at 11-26 days post cis-platinum therapy. In contrast to animal studies, the distal tubule and collecting duct appear to be most damaged, while both groups show a similar lack of abnormal glomerular pathology[309].

The mechanisms responsible for the observed structural alterations are not known. It has been observed that platinum levels are greatest in the cortical regions of rat kidney[307,310] and it is here that certain secretory functions, including those for heavy metals, take place. Therefore, somewhat selective accumulation via this mechanism may result in damage to the secretory cells. Renal pathology may also be related to cis-platinum binding to the proximal tubule microvilli via the free sulphydryl groups[307]. A reduction in

renal blood flow may contribute since the outer stripe should be more susceptible to hypoxic conditions[306].

c) Consequences: In animals, a variety of renal excretory functions are impaired. In rats, cis-platinum has been shown to increase urine volume, while decreasing osmolality, indicating the drug influences renal concentrating ability. In high doses (10 mg/kg) urinary pH was transiently reduced, and glycosuria with hyperglycemia was detected. Electrolyte imbalance was also evident, consisting of excessive potassium excretion and hypokalemia[311]. Hypomagnesemia has been observed in cis-platinum treated patients due to renal magnesium wasting[311,312] with the secondary development of hypocalcemia[311]. These two disturbances are likely responsible for the precipitation of tetanic spasms in some patients[313]. All of these changes are related to the pronounced effect of the drug on the tubule structure where reabsorption and thus body retention of these substances normally takes place.

In vitro studies have shown that high concentrations of cis-platinum inhibit the renal tissue accumulation of para-aminohippurate and tetraethylammonium ions[303]. This property is observed to a smaller extent in vivo since the available platinum levels would be considerably lower. There appears to be a generalized depression of renal metabolic function, with evidence from in vitro inhibition of the enzyme ATPase[314]. Functional impairment may also be a consequence of reduced glomerular filtration rate due to renal insufficiency. A reduction in renal blood flow may be mediated via a decreased absorption of sodium chloride and an increased salt delivery to the distal tubule, which stimulates the juxtaglomerular apparatus,

causing activation of angiotensin II which constricts the glomerular arterioles[315].

d) Reduction: Numerous attempts have been reported in the literature with regards to minimizing the nephrotoxicity associated with cis-platinum treatment. These methods generally involve the induction of diuresis or pharmacological complexation of the nephrotoxic platinum species but other approaches have also been reported.

Cis-platinum nephrotoxicity cannot be successfully prevented by reducing the rate of administration or fractionating the dose over a period of days unless these methods are used in conjunction with induction of diuresis and/or hydration[315]. Several studies with rats have indicated that hydration alone or in combination with furosemide and/or mannitol effectively reduce the observed degree of renal dysfunction[39,314,317-320], although agreement is not universal[321]. In humans, the apparent benefits of several combinations of hydration with or without mannitol or furosemide diuresis are somewhat variable. Hydration protocols alone have been shown to prevent cis-platinum renal damage[322,323] and the addition of mannitol to this regimen is of no[324] or slight[316,325,326] benefit. The use of hydration plus mannitol and furosemide has not been shown superior to the use of fluids and either diuretic alone[248]. Similarly, there is no difference between the use of hydration and mannitol versus hydration and furosemide[246,248]. The use of these techniques results in a decrease of renal platinum concentrations via dilution and usually an increase in excretion, both of which contribute to the reduction in nephrotoxicity. In addition, mannitol administration may react with

cis-platinum[115] to form a less nephrotoxic complex which is readily excreted.

The use of furosemide and mannitol as diuretics does not apparently alter the tissue distribution of the drug in rats, although excretion is increased using mannitol. Thus, the expected therapeutic activity may also be unaffected[47]. However, both cis-platinum and furosemide affect the renal transport of para-aminohippurate in vitro[314], and may represent additive toxicity if used in these combinations.

Various agents are capable of reacting with and potentially detoxifying cis-platinum. Available sulphur ligands react rapidly and covalently with cis-platinum and thus compounds containing these groups have been extensively investigated. In vitro studies have shown that thiourea[327,328], methionine and thiouracil[327] in high concentrations can reduce the toxic effects of cis-platinum although cysteine did not[327]. In vivo, thiourea and thiosulphate are the most potent inhibitors of cis-platinum lethality with propylthiouracil and methiomazole intermediate and cysteamine like N-acetyl-L-cysteine possessing little or no protective effect[329,330]. Clinical usage is likely prohibited since thiosulphate reduces the antitumor activity of the drug as well[331]. A phosphorothioic acid derivative has been found to reduce the nephrotoxicity without altering the therapeutic activity.[332] Penicillamine has also been shown to be effective in both respects in animals but has not proven effective in ameliorating renal toxicity in man[333]. These agents exert their protective effects by direct action on unreacted cis-platinum since they are only effective when administered before drug dosing[331,334]. In rats and

mice, diethylthiocarbamate decreases the degree of nephrotoxicity of cis-platinum[335] and has no effect on the antitumor response[332,336]. Since this compound may be administeed up to four hours after cis-platinum, it may be acting by the removal of prebound drug which could be attached to renal tubule sulphydryl groups[332].

Probenecid is a known inhibitor of tubular transport systems and reduces the renal pathology and functional inhibition of cis-platinum treated rats[337]. No effect on immunosuppressive[338] or antitumor properties have been noted[337]. This suggests it may prevent accumulation of the toxic cis-platinum species within the renal tubules[300]. Orgotein (zinc-copper superoxide dismutase) has been shown to reduce the nephrotoxicity of cis-platinuum in rats, possibly by reducing oxygen radical involvement in the destruction process, but has not been tested clinically[339]. It has been suggested that specific prostaglandins are involved in the process and that others may be helpful in reducing these effects[300].

Pretreatment of rats with urinary acidifiers reduces the cumulative excretion of cis-platinum and enhances kidney retention of the drug while an increase in urinary pH enhances renal excretion. However, the cis-platinum induced renal pathology after both types of pretreatment is apparently similar despite the impaired handling of the drug during acidosis[340]. Administration of cis-platinum to rats in a hypertonic saline solution reduces its toxicity while the antitumor properties are retained. This procedure produces faster plasma clearance, reduced protein binding and lower tissue concentration[240]. Hypertonic saline infusions appear promising and await clinical evaluation. Attempts to take advantage of the

circadian rhythm of renal performance have shown that cis-platinum administration near periods of maximal urinary volume minimizes kidney damage relative to other daily circadian phases[341,342].

6. Miscellaneous toxicity

A few patients have experienced hypersensitivity or allergic reactions, opthamologic toxicity, liver damage, cardiotoxicity, hypertension and local fibrosis after cis-platinum administration[40,61].

LITERATURE SURVEY - GALLIUM-67

I. Introduction

The element gallium was discovered by Lecoq de Boisbaudran in 1875 and named in honour of France[343]. Many of the early investigations on the biological behavior of gallium were carried out by H.C. Dudley and his co-workers in the 1949-52 year period[344,345,346]. This group first noted the affinity of radiogallium-72 for osseous tissue and suggested its application as a bone scanning agent[347]. In 1969, with the production of carrier free ^{67}Ga, came the serendipitous discovery by C.L. Edwards and R.L. Hayes that when injected intravenously as the citrate external scintimaging of soft tissue tumors was possible[348,349]. Since that time, the ^{67}Ga-citrate tumor scan has become a widely practiced and accepted diagnostic test. Concurrently, there continues to be a large scientific effort to define its biological behavior under various conditions with hopes of establishing a cellular mechanism of tumor uptake and improving its clinical effectiveness.

II. Production and Nuclear Properties

Only two of the 23 known isotopes of gallium are stable (^{69}Ga 60.1% and ^{71}Ga 39.9%)[350]. Radiogallium in the form of ^{72}Ga, ^{68}Ga and ^{67}Ga have been used in nuclear medicine but only the latter two are of current interest. ^{72}Ga is reactor produced from natural gallium and ^{68}Ga is the daughter of the long-lived cyclotron-produced ^{68}Ge. The unsuitable photon energies and the carrier effect associated with ^{72}Ga production have made it obsolete while the short half-life of ^{68}Ga has precluded its use as a tumor localizing agent. Gallium-68 does, however, hold promise as a positron-emitting label for other compounds which may possess the required tumor localizing properties.

The production of ^{67}Ga has been accomplished using several nuclear reactions. Charged particle bombardment of natural or enriched zinc in a cyclotron has been shown to yield ^{67}Ga via $^{66}Zn(d,n)$, $^{67}Zn(p,n)$ and $^{68}Zn(p,2n)$ reactions. The conditions of these irradiations and the reported yields have been summarized in Table 6.

Zinc metal, even when electroplated on certain other support metals, tends to flake under high beam current irradiations[351]. The greater stability of copper targets under such conditions led to their use for ^{67}Ga production via $^{65}Cu(\alpha,2n)$ and $^{65}Cu(^{3}He^{+},n)$ reactions. The former process has a high cross-section (10^{3} b at 28 MeV and 10^{2} b at 16 MeV) in comparison to the latter reaction (13 b at 12 MeV)[351] and thus explains the large yield differences for these reactions shown in Table 6.

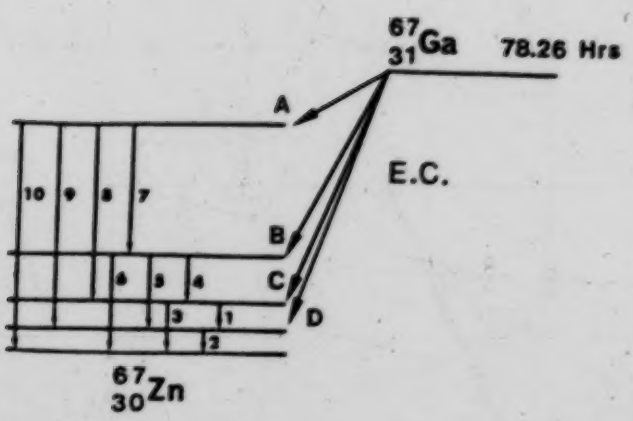
The major radionuclidic impurity present after bombardment in the majority of cases is ^{66}Ga especially when using natural zinc targets (^{66}Zn 27.8%) where $^{66}Zn(p,n)$ and $^{66}Zn(d,2n)$ reactions occur. This necessitates a post-irradiation cooling period to minimize the unfavourable contribution of ^{66}Ga to available ^{67}Ga activity.

The preferable production routes are those which make use of enriched zinc targets which increase the ^{67}Ga yield and suppress impurity production.

There have been three methods reported for the separation of radiogallium from the target matrix. The oldest method involves repeated solubilization with acid and precipitation by various bases followed by ion exchange chromatography[351]. A more successful approach utilizes the rather specific extraction of Ga(III) ions from strong

hydrochloric acid solutions by di-isopropyl ether. Dissolution of the target in 12 M HCl followed by washing with the immiscible ether has been shown to almost completely separate ^{67}Ga from zinc contaminants. Stripping the ^{67}Ga back into 0.2 M HCl and evaporating to dryness leaves $^{67}GaCl_3$, while the target material is easily recovered from the initial acid solution[353]. A third, more specialized technique, involves the use of an electrolysis cell. It has been shown that with proper assessment of current changes and pole materials, the effective separation of ^{67}Ga from zinc targets may be accomplished[354].

^{67}Ga decays by electron capture (100%) to one of four excited states of ^{67}Zn and immediate transitions result in the emission of 10 gamma rays[350,355], as shown in Figure 3. The gamma-ray constant is 1.6 R/mCi-hr at one centimeter and the half-value layer for lead is 0.04 mm[356]. The half-life of 78.26 hours[355] is sufficient for distribution and clinical use of the isotope.



TRANSITION (E.C.= Electron Capture)	ENERGY (kev)	ABUNDANCE (percent of decays)
E.C. A	130	0.37
E.C. B	606.5	27
E.C. C	815.4	25
E.C. D	906.7	48
gamma 1	91.27	3.07
gamma 2	93.31	38.3
gamma 3	184.58	20.9
gamma 4	208.95	2.37
gamma 5	300.22	16.8
gamma 6	393.53	4.7
gamma 7-10	400-900	0.3

Figure 3. Decay scheme and emission characteristics of Ga-67.[330,335]

Table 6. Summary of reported production parameters for ^{67}Ga.

Reaction	Target	Particle Energy (MeV)	Beam Current (μA)	Yield (μCi/μA-hr)	Impurities	Ref
$^{66}Zn(d,n)$	Zn	8	200	30	^{69m}Zn, ^{65}Ga	357
$^{66}Zn(d,n)$	Zn	16	200	280	^{66}Ga	358
$^{66}Zn(d,n)$	Zn	-	-	100	^{65}Ga, ^{68}Ga	359
$^{66}Zn(d,n)$	^{66}Zn(90%)	16	-	946	^{66}Ga	354
$^{67}Zn(p,n)$	Zn	15	-	77	^{66}Ga	357
$^{67}Zn(p,n)$	^{67}Zn(89.55%)	21	180	167	^{66}Ga	360
$^{68}Zn(p,2n)$	Zn	21	233	330	^{66}Ga	360
$Zn(p,2n)$	^{68}Zn(98.5%)	22	350	1600	^{66}Ga(5%)	353
$^{65}Cu(\alpha,2n)$	Cu	15	-	0.13	-	357
$^{65}Cu(\alpha,2n)$	Cu	30	500	160	^{66}Ga	351
$^{65}Cu(^{3}He^{+},n)$	Cu	23	-	1.1	^{65}Zn, ^{66}Ga, others	357

II. Chemistry

The chemistry of gallium is similar to aluminum due the similar electronic and physical properties of the two elements. Gallium readily alloys with other metals and reacts to form a variety of inorganic compounds and organic complexes. These would include simple salts, oxides, complexes with organic acids (such as lactic, tartaric, oxalic and acetic) and nitrogenous compounds[253].

A brief summary of some simple inorganic compounds and more specifically with gallium citrate as they relate to radiogallium

chemistry will be presented.

A. Simple Inorganic Forms

There are three oxides of gallium and the most stable is the β conformation of the sesquioxide (Ga_2O_3). The oxides do not exist in aqueous solution since they are converted to hydroxides. The most prevalent of the four reported hydroxides which exist in alkaline solutions is the simple trihydroxide [$Ga(OH)_3$]. This hydroxide is present from pH 3 but is predominate after pH 4.5[361]. Other simple hydroxides become more prevalent at narrow pH ranges [$Ga(OH)^{+2}$ at pH 2.8-3.5; $Ga(OH)_2^+$ at pH 3.5-4.5][362]. The pH of precipitation of $Ga(OH)_3$ from basic solutions has been found to be dependent upon the concentration of gallium, the solution temperature and the presence of specific anions or polyhydric alcohols. In the neutralization of 0.01 M $GaCl_3$, precipitation of $Ga(OH)_3$ begins at about pH 3.4-3.5, is complete at about pH 6.7 and dissolves at about pH 9.6. At least part of the $Ga(OH)_3$ is apparently in a colloidal state. The alkali soluble gallates ($Ga(OH)_4^-$) are formed by further addition of base to a pH 9-10[361]. The reported neutralization of radiogallium chloride showed the formation of three chromatographically distinct products in the pH range 2.5-10.6[363].

Gallium phosphate and its hydrated forms have been prepared by the neutralization of $GaCl_3$ to pH 5-5.4 in the presence of phosphate ions, and a 1:1 polyphosphate complex has also been reported[363].

B. Gallium Citrate

The reaction of gallium and citric acid in equal amounts in

acidic solution produces a 1:1 citrate:gallium complex. The exact composition of this product has not been determined but has been shown to be a function of both pH and reagent concentrations.

A study published in 1967 reported the following conversion takes place as a function of pH[364].

$$GaOC_3H_4(COOH)_3 \underset{pH \sim 2}{=\!=} Ga(OH)_2OC_3H_4(COOH)_3$$

$$Ga(OH)_2OC_3H_4(COOH)_3 \underset{pH \sim 3}{=\!=} Ga(OH)_2OC_3H_4(COO^-)(COOH)_2$$

$$Ga(OH)_2OC_3H_4(COO^-)(COOH)_2 \underset{pH \sim 4}{=\!=} Ga(OH)_2OC_3H_4(COO^-)_3$$

These findings were based on infrared absorption data of the solid complexes at citrate:gallium ratios of 1:1[364]. In this case, the hydrolysis of the gallium ligand occurs before the carboxyl hydrogens are neutralized. It is difficult to understand why the least acidic proton of the hydroxide is replaced by gallium in preference to those of the carboxyl groups.

In contrast, in the narrow pH range 1.3-3.0, the following reactions of 1:1 citrate:gallium mixtures have been reported[365].

$$Ga^{+3} + HOC_3H_4(COO^-)_3 + iOH^- =\!= Ga(OH)_iHOC_3H_4(COO^-)^{-i}$$

$$i = 0,1,2,3,$$

More specifically, at pH < 2 $GaHOC_3H_4(COO^-)_3$ and $[Ga(OH)HOC_3H_4(COO^-)_3]^{-1}$ are formed, the latter predominating. In the pH range 2-3, $[Ga(OH)_2HOC_3H_4(COO^-)_3]^{-2}$ is also formed. When the citrate:gallium ratio is reduced to 0.5, the trihydroxo complex has been observed.

The reaction at the carboxyl groups is similar to that reported for other organic acids[243] and for In-citrate[364]. Other reports present evidence to support the anionic nature of the gallium citrate complexes[366,367]. In addition, polymer formation of the type $[Ga(OH)_2HOC_3H_4(COO^-)_3]^{-6}_3$ has been detected[368].

Most recently, potentiometric and NMR (nuclear magnetic resonance) studies of this reaction have indicated that in mildly acidic to neutral solutions two types of complexes can be formed depending upon the stoichiometry. With citrate in excess a 2:1 citrate:gallium complex predominates over a 1:1 form, while when gallium was at least 50% of the citrate levels, a polymeric form was most abundant. It was suggested the polymeric form was a small oligomer of the 2:1 complex. The addition of more base causes dissociation of the polymer into smaller units which further decompose to gallate and free citrate at pH 9-10[368,369,370].

All of these studies dealt with gallium and citrate concentrations in the 0.001 - 1.0 M ranges. However, radiogallium-67 at a level of 2 mCi/ml has a gallium concentration of approximately 10^{-7} M, and is essentially in a no-carrier-added form. In normal ^{67}Ga-citrate radiopharmaceutical doses the citrate ranges from 2-25 mg/ml and provides a citrate:gallium ratio of 10^5 to 10^6:1. Even under these condictions, the chemical and/or physical form of ^{67}Ga-citrate may be altered by pH[371] and citrate concentration changes[372] and detected by chromatographic separation. At neutral pH and citrate levels greater than 10 mg/ml, greater than 90% of the ^{67}Ga activity has been shown to exist as a citrate complex. This value decreased to 60% at 1 mg/ml and less than 10% at 0.2 mg/ml. At 2

mg/ml citrate levels, greater than 90% was found at an R_f corresponding to ^{67}Ga-citrate when the pH was less than 6, with progressively more $Ga(OH)_3$ and gallate being formed at higher pH values[371]. We may speculate that the ^{67}Ga-citrate present at low pH or high citrate (> 10 mg/ml) at neutral pH is a polymeric form possibly containing more than one citrate per ^{67}Ga ion which is soluble at these concentrations.

The biological consequences of ^{67}Ga chemistry and formulation will be discussed in a later section.

IV. Biological Behavior

A. Distribution

The tissue localization of ^{67}Ga in laboratory rodents has been reported in numerous studies. Tracer uptake, as a function of time after injection of ^{67}Ga-citrate, follows a similar pattern in both rats and mice. Concentrations in the liver, spleen and kidneys show peak activity from 24-48 hours with subsequent monoexponential washout. Other tissues such as lungs, testicles, gut, heart and plasma peak within a few minutes and are washed out with either a single or double exponential pattern[373], indicating the rate of uptake is negligible compared to the rate of elimination.

In man, an intravenous injection of ^{67}Ga-citrate has been shown to quickly bind to plasma proteins and distribute throughout the body[374].

A qualitative knowledge of the normal pattern of localization in man at 48-72 hours is imperative for proper scan interpretation. In the head and neck region, intense uptake is noted in the nasopharnyx,

the lacrimal and salivary glands. ^{67}Ga typically concentrates in the bony structures of the thorax, sternum, ribs, clavicle, spine, scapula and skull. The most intense localization can be observed in the abdomen, specifically the spleen and liver. Uptake in the colon can generally be interpreted by the recognizable linear pattern of the large bowel. Tracer accumulation by the hip joint, lumbar spine, sacrum, ilium, ischium and occasionally external genitalia can also be observed. Intense uptake at the epiphysis of long bones in children or adolescents is normal[374,375,376]. Thus, localization in most tissues and organs can be expected, although low levels are observed in the brain, cerebrospinal fluid and muscles[377].

Quantitatively, the highest concentrations of the tracer occurs in the spleen, kidney cortex, adrenals and bone marrow with each accounting for 3-5% of the administered dose per kilogram. Considerable accumulation has been shown in the liver, kidney medulla, bone, gut and lung with 1-3% uptake. Other organs and tissues generally contain less than 1%, however, wide fluctuation in all values has been observed[378]. Tumors have been found to take up a variable amount of ^{67}Ga depending in part on histological type and degree of necrosis. When tumor localization was less than 5% of the administered dose per kilogram, detection is reportedly difficult[379].

B. Excretion

Excretion measurements of ^{67}Ga after intravenous doses of tracer ^{67}Ga-citrate show some similarities between species. Whole body elimination in man occurs with half-lives of 1.3 and 25.5 days, the majority of activity from the slow phase[393]. Estimates of the long

term biological half-life of ^{67}Ga in mice have been reported at 15.5 and 17 days[394,395].

Urinary excretion in man usually represents 10-15% of the dose in the first 24 hours, 3-5% of the dose in the next 24 hours and again about 3-5% from 2-3 days[379,396]. The urinary ^{67}Ga may be in the form of the intact citrate, as $^{67}Ga(OH)_3$, $^{67}Ga(OH)_4^-$,[397] or phosphate[398].

The elimination of ^{67}Ga via the human gastrointestinal tract accounts for 9-15% of the administrated dose after a week[349,378,399]. In rats, about 5% of the dose is excreted in the feces in each of the first 3 days[400]. Estimates of the source of this activity have shown it to be secreted by the intestinal mucosa while only about 20% of the total is derived from bile[401], a figure which may be much higher in humans[402].

C. Pharmacokinetics and Plasma Protein Binding

^{67}Ga-citrate pharmacokinetics have studied in several species and in man. The ^{67}Ga activity plasma clearance data in man was found to best fit a 3-compartment model described by a three exponential equation. The phase half-lives were found to be less than one hour, between 2-10 hours and 2-4 days for the fast, intermediate and slow phase respectively[380,381] Thus, within a few hours after intravenous administration, most the injected activity has left the blood and by 2-3 days, less than 5% remains[381].

Blood clearance of ^{67}Ga-citrate in mice is very rapid initially. Approximately 25% of the dose is present in the circulation after 30 minutes and only about 1% remains after 24 hours[382,383].

Studies conducted using both *in vitro* and *in vivo* samples have

demonstrated the association of ^{67}Ga with plasma components[384,385]. Shortly after ^{67}Ga-citrate administration, 85% of the activity becomes protein bound and is virtually 100% bound in samples taken after 24 hours. About 70% of the ^{67}Ga was thought to be loosely associated with albumin but electrophoretic studies revealed binding only to transferrin and haptoglobulin[386]. These electrophoretic results have been disputed[387] since the electric field distorts the actual binding pattern[384]. However, the association of ^{67}Ga with transferrin has been substantiated by several other methods[388]. The exact nature of the gallium-transferrin interaction has not been defined. Studies with stable gallium have shown the protein has 14 binding sites for gallium, when present as a colloidal form of the citrate[381], and 2 specific binding sites[389] and about 20 total binding sites[405] for the hydrated Ga(III) ion.

Plasma protein binding *in vitro* can be affected by specific incubation conditions. Citrate concentrations in the range of 1-2 mg/ml and pH <6.5 reduce ^{67}Ga binding[371]. The addition of iron[390], scandium[391] and carrier gallium salts[384] have also been shown to inhibit this process. Citrate ions do not affect the *in vivo* binding of ^{67}Ga-citrate[371] but the presence of specific cations, especially iron, have been shown to compete with ^{67}Ga for the plasma protein binding sites[392].

D. Subcellular Localization

1. Specific Organelle Uptake

Early studies with ^{67}Ga at the cellular level revealed the majority of activity was localized within the cytoplasm[403].

Subsequently, it was revealed that electron-dense heterogenous bodies 0.2-0.04 μm in size contained most of the ^{67}Ga, and were morphologically identified as lysosomes, phagolysosomes, or peroxisomes[404]. Other work reported the association of ^{67}Ga with the sedimentation bands of liver cells containing the highest acid phosphatase, β-glucosamidase and aryl sulphatase and further established the link with lysosomal uptake[406-411]. There may also be clinical evidence of lysosomal uptake, such that tracer accumulation in amyloidosis could be related to increased activity of these organelles[412]. The reported prominence of the tracer in the soluble portion[413-415] of liver and tumor cells has been attributed to the fragility of lysosomes[416] and indeed a differential disruption of these organelles has been demonstrated[409]. ^{67}Ga activity associated with the nuclear fraction[407] has been shown to be likely due to procedural problems[417].

Both the light and heavy forms of lysosomes have been shown to accumulate ^{67}Ga[409,418] and uptake in rough endoplasmic reticulum[409,419] and peroxisomes[420] have also been identified.

2. Intracellular Binding

The association of ^{67}Ga with intracellular macromolecules has been demonstrated in liver and tumor homogenates. However, the identity and properties of such a complex are not known. Several investigations have concluded the substance is a glycoprotein with a molecular weight of $4.5\text{-}5.5 \times 10^4$ and a 50% protein composition[421,422]. Saturation of this molecule with small amounts of stable gallium reportedly shifts the ^{67}Ga to a $1\text{-}1.2 \times 10^5$ molecular

weight fraction which possesses a higher binding capacity[421]. Others have confirmed the presence of a 45,000 molecular weight and a heavier component binder of intracellular ^{67}Ga[423].

Identifiable substances have also been implicated. A transferrin-^{67}Ga complex has been detected in tumor cells by immunoprecipitation shortly after intravenous administration of ^{67}Ga-citrate[423]. Indirect evidence for an intracellular ^{67}Ga-ferritin association can be suggested, since patients whose ^{67}Ga-citrate scan showed positive tumor uptake had greater serum ferritin levels[424]. Some degree of ^{67}Ga ferritin binding has been observed in tumor cell homogenates[381] and in rabbit hepatocytes[425] but not in rat liver homogenates[426]. Lactoferrin has been reported to bind ^{67}Ga more avidly than transferrin[427] and is found in certain tissues, fluids and tumors which show ^{67}Ga uptake[427-430].

V. Clinical Applications

A. Dosage, Administration and Scanning

A general approach to radiopharmaceutical imaging is to achieve optimal sensitivity coupled with minimizing the detected scatter. Of the four main gamma emissions of ^{67}Ga, the 93 KeV photopeak is most abundant and most efficiently detected and therefore of primary importance to the statistics of imaging. However, it suffers the most from scattered radiation intererence. The two higher energy photons at 185 and 300 KeV are sufficiently abundant and detectable to be included as well as being less influenced by scatter. Depending upon the instrument, several window settings are capable of producing adequate images. Those with a single pulse height analyzer are best

suited to a 20% window around the 93 KeV peak. When multiple windows are available, smaller windows around each of these peaks increased the overall efficiency and minimizes the effects of scatter[431].

If a choice of instrument is available a LFOV (large field of view) Anger camera is preferred to a rectilinear scanner[432], and with a slight sacrifice in sensitivity a partial tomographic display may be obtained with the Anger tomoscanner[431].

The use of a collimator which does not allow penetration of the 384 KeV photons is preferred since all photons are attenuated equally and scatter is minimized. This usually means the use of a medium to high energy collimator[431].

Initial clinical studies using ^{67}Ga-citrate advocated the use of bowel cleansers to minimize abdominal interference. However, recent studies have shown several bowel preparations to be of limited or no value in this regard[433,434]. Others have reported castor oil to be more effective than milk of magnesia (30 ml) or cascara and some reduction in colonic activity was demonstrated for a three day high fibre diet[435]. The effectiveness of enemas has not beem reported but are generally administered if significant bowel activity is persistent at the 48 hour scan[431].

^{67}Ga-citrate is administered as a bolus intravenous injection at a dosage of 3-15 mCi, with scanning normally performed 48 or 72 hours thereafter.

B. Clinical Uses

1. Malignant Diseases

When used at the appropriate doses and with optimal imaging

technique, the ^{67}Ga-citrate scan has been shown to be of value in the detection of numerous malignant diseases. Various aspects of patient care including staging procedures, treatment responses, follow-up tests for recurrent disease, and detection of occult tumors have been accomplished using this technique. The availability and clinical promise has resulted in extended studies to determine those conditions where evaluation would be aided by a ^{67}Ga-citrate scan. These investigations have pointed out the non-specific nature of ^{67}Ga accumulation and redefined its role in the detection of malignant disease. A brief summary of the most current literature and published reviews on the clinical utility of the ^{67}Ga-citrate scan will be presented.

Hodgkins Disease: The use of the ^{67}Ga-citrate scan has been shown to correctly indicate sites of nodal involvement of this disease in about 70%[436] of adult cases and between 50[427]-80%[428] of pediatric patients. In adults, this figure has been shown to vary depending upon the histologic type, size and location of the lesion as well as whether the patient is undergoing treatment[439]. It is apparently most sensitive for non-lymphocyte predominant types, progressively better for lesions between 1-5 cm, for mediastinal involvement and for untreated patients[436,439]. When used in conjunction with lymphangiography, higher overall sensitivity can be obtained due to the greater ability of ^{67}Ga to identify areas of extranodal involvement combined with greater nodal sensitivity of the radiographic technique[440]. The ^{67}Ga-citrate scan is thus of great value in reaching most therapeutic decisions involving Hodgkin's diseased patients[441].

Non-Hodgkins Lymphoma: The sensitivity of the ^{67}Ga-citrate scan in non-Hodgkins lymphoma has been reported between 52[442]-65%[443]. A similar dependence on histology, size, location and treatment as with Hodgkins lymphoma was observed. Generally, large extranodal histiocytic lesions in patients with no prior treatment showed the highest true positive rates[442,443,444].

Although lymphangiography has been shown to be more effective to detect masses in the pelvic-abdominal region[442], the results of both tests are highly correlated[445]. Thus, the comparative utilization of both techniques may not be warranted for routine use[442], but confined to patients who show tumor accumulation on the initial scan and return for detection of suspected recurrance[446].

Burkitts Lymphoma: In studies involving limited numbers of patients, a 100% sensitivity figure has been reported[443,446]. This indicates the ^{67}Ga-citrate scan should be valuable in the evaluation of patients with this malignancy.

Leukemia: ^{67}Ga possesses a greater affinity for granulocytes than for lymphocytes *in vitro* and *in vivo*[447]. Thus, ^{67}Ga has been shown to be taken up in the extramedullary sites and bone marrow of acute lymphocytic, acute myelocytic, chronic granulocytic but not in lymphocytic leukemia patients[447,448]. The ^{67}Ga-citrate scan may be thus useful in specific cases for the detection of leukemic masses[448,449].

Malignant Melanoma: The reported sensitivitiy of the ^{67}Ga-citrate scan for the detection of this condition is 54[450] - 69%[451]. Lesions greater than 2 cm, especially in the bone, were most reliably detected[450] and often unsuspected metastases can be visualized[436].

Breast Tumors: The relatively low detection rate (54%)[452] of the ^{67}Ga-citrate scan for primary breast lesions plus the normal variability of breast uptake makes this technique inferior to other methods for this purpose[436].

Hepatoma: ^{67}Ga-citrate imaging has been successfully used in combination with radiocolloid scans in the differentiation of hepatic tumors from cirrhosis, either by visual comparison[436,449] or by digital subtraction techniques[453]. High uptake of ^{67}Ga in an area which showed poor accumulation of colloid is the most important diagnostic criteria. About 90% of hepatomas, but only 10% of cirrhotic lesions take up the ^{67}Ga[454].

Generally, ^{67}Ga liver uptake can be related to the degree of vascularity, since moderately vascular lesions showed more accumulation than normal or hypovascular areas[455]. Increased uptake is not solely indicative of tumor because hepatic abscesses and hepatic metastases from other tumors may take up the tracer[449]. Positive correlation of ^{67}Ga-citrate images with α-fetoprotein levels have been reportedly good[456] or non-existent[455].

Bone Tumors: Primary skeletal lesions are more effectively imaged by the bone scan and radiographs have been shown more sensitive for soft tissue metastases[457]. Thus, the ^{67}Ga-citrate scan is not particularly useful for skeletal tumors.

Genitourinary Tumors: Renal tumors have a reported sensitivity of about 50%[458] and detection is hindered by variable kidney uptake in normal subjects[454]. Limited patient studies with cervical, ovarian, prostate, bladder and uterine neoplasia have shown similar low sensitivity (28%, 54%, 60%, 41% and 50% respectively)[458].

Several reports have indicated that although ^{67}Ga-citrate imaging does not accurately locate primary testicular tumors, it is of value in the detection of metastases, in staging, and suspected recurrance[459-462]. Most testicular tumor types show at least some sensitivity[458], with the best results obtained in embryonal (74%)[29] and seminomatous (87%)[460] varieties.

Gastrointestinal Tract Tumors: Most GIT cancers are not well visualized in ^{67}Ga-citrate images, with sensitivities for pancreatic, colonorectal and stomach being reported at less than 50%[436,458]. Scan interpretation is again hindered by normal variation of uptake in the abdominal area, but in certain clinical situations[464] such as the detection of tumor recurrance after primary colonorectal excision[465], it may be of value. In a small study, primary esophageal tumors have been poorly detected but metastases were visualized much better[466], and await further evaluation.

Head and Neck Tumors: The most recent report on the use of ^{67}Ga-citrate for the detection of tumors of the head and neck indicated a 56% sensitivity[467], while previous figures were in the 38-61% range[458]. The technique cannot be considered for routine detection in this area because of these figures and the fact that negative scans have not been shown to reliably rule out the presence of tumor[467]. Specific organs such as the thyroid show a smiliar low detection rate and are hindered by the uptake of ^{67}Ga by benign thyroid conditions[468].

2. Inflammatory or Infectious Conditions

Although ^{67}Ga-citrate scanning was initially investigated as a

tumor localizing agent, it soon became evident that focal sites of inflammation and infection were also visualized. Once this feature was recognized, studies were initiated to develop the possible clinical applications. Several definite roles for the technique utilizing this ability have been assessed.

Abdominal Area: Current reviews of the use of ^{67}Ga-citrate for the detection of intra-abdominal lesions have reported an overall sensitivity and specificity of 90%[469,470]. ^{67}Ga has been shown to accumulate not only in well-formed abscesses but also phlegmon and peritonitis, where other modalities (such as computed tomography and ultrasound) are less useful[470,471]. Thus, the ^{67}Ga-citrate scan is preferred when the location of the site of the infection is unclear and no infectious foci in this area can be demonstrated by other techniques.

Bone and Joint: Radiogallium has been shown to be a useful adjunct for the detection of suspected inflammatory bone lesions (such as osteomyelitis) when the bone scan is negative[469,471] or in neonates where the bone scan has been shown to be somewhat insensitive[36]. In addition, even with a positive bone scan, the ^{67}Ga-citrate image can be useful to differentiate acute inflammatory lesions of the bones and joints from more chronic cases[470]. Phosphate bone scans tend to remain positive in areas of osteomyelitis, while ^{67}Ga accumulation diminishes with therapeutic measures and may thus serve as a guide to therapy[472].

Lung: Virtually all pulmonary lesions which cause inflammations have shown ^{67}Ga uptake. As such, it has claimed clinical benefits from: the differentiation of sarcoid masses from malignant ones; the

differentation of pulmonary infarcts from infections; early detection of microbial infection and pneumoconiosis; and the assessment of the effectiveness of treatment in idiopathic pulmonary fibrosis[469-471].

Kidney: The uptake of ^{67}Ga in non-neoplastic renal lesions includes pyelonephritis, acute tubular necrosis, renal vasculitis, interstitial nephritis, renal abscesses, amyloidosis, nephrolithiasis and transplant rejection[412,469,472,473]. In general, bilateral renal uptake that persists significantly after 24 hours requires further evaluation[472].

Miscellaneous: Cases involving ^{67}Ga uptake in bacterial endocarditis, myocardial abscesses[472], thyroiditis, Duchenne muscular dystrophy[471] and cranial inflammatory conditions[471] have also been reported. The tracer accumulation at injection sites or surgical wounds are usually of minor clinical significance[471].

Early comparison of ^{67}Ga-citrate with radioindium labeled white blood cells (In-WBC) for visualization of occult sepsis revealed acute infections are more readily detected with In-WBC, but in chronic low grade infections ^{67}Ga-citrate may be somewhat more effective[474,475].

VI. Factors Affecting Localization

A. Age

Early animal studies documented a relationship between age and the degree of tissue uptake. Five week old rats had less ^{67}Ga accumulation in liver, kidney, lung, spleen and blood and more in bone compared to their twenty week old counterparts[416]. However, this effect has not been observed in humans[378], with the exception of increased thymic and epiphyseal deposition in children[431].

B. Gender

The male gender of at least three species of rats have been shown to have increased ^{67}Ga uptake in spleen, bone marrow and muscle, while females showed greater uptake in blood, kidney and liver[416,476]. When male rats were castrated distributions resembling those in females were observed and subsequent administration of testosterone produced accumulation approaching those observed in normal males. Testosterone also produced 'normal male' distributions in females and estradiol similarly induced 'normal female' patterns in males[477]. The overall whole body retention of ^{67}Ga in humans has been reported to be longer in females than in males[393].

C. Pregnancy and Lactation

A hormonal influence may also be involved in the tissue accumulation of ^{67}Ga during pregnancy and lactation. Concentrations in the embryonic tissue of mice varied depending upon the time of gestation, reaching a maximum of 13% of the dose on day 9. ^{67}Ga was located in the decidua, fetal membranes and limb buds at 8-13 days,

but by day 14 most appeared in the skeleton[478]. Within rabbit mothers, uterine tissues showed a marked uptake by day 5, but by 7-8 days, the blastocyte and implantation sites were the major gestation associated sites with ^{67}Ga activity[479]. By 15-28 days, the placental uptake predominated and was clearly visible by external scanning[478,480]. Blood, spleen and kidney activity of the mothers was reduced especially at later time periods[479].

Breast uptake has been observed in several non-neoplastic conditions such as galacterhea[481] and in lactating females[482,483,484]. The external visualization has been shown to be due to the presence of both glandular and fluid ^{67}Ga activity[427]. In dogs at 48 hours after ^{67}Ga-citrate administration, these contribute about equal amounts[427], but at 24 hours in the rabbit about 90% was found to be associated with the milk[485].

D. Nutritional Status

Although emanciated patients showed no qualitative differences in ^{67}Ga-citrate scans[378], studies in rats have shown 24 hour food withdrawal results in increased liver, spleen, kidney, bone marrow and blood concentrations as well as an increase in whole body retention[486].

E. Formulation

Radiogallium may be prepared in several chemical forms which are suitable for injection. However, only specific ligands are suitable for use as tumor-localizing radiopharmaceuticals. The chemical nature of other radiogallium compounds has dictated their

utility, namely colloids for liver scintigraphy[487], magnesiuum polymetaphosphate for renal scanning[488], and phosphates for bone[489] or myocardial imaging[490].

The search for ligands which would effectively solubilize radiogallium at physiological pH eventually led to citric acid, primarily because of its ease of complex preparation[491]. Other chelate forms such as nitrilotriacetate, salicylate and lactate have been shown to result in similar tissue distributions to ^{67}Ga-citrate[1,392].

The isotope may also be administered as the simple trichloride, provided the pH is acidic enough to prevent colloid formation[382,416].

The citrate concentration in the injection solution or *in vivo* has no effect on the biological handling of ^{67}Ga-citrate[1,371,394], in spite of the observed chromatographic differences between preparations with varying amounts of citrate[371,372]. However, large quantities of citrate may decrease the degree of *in vitro* protein binding[372].

F. Iatrogenic

1) Irradiation

The whole body irradiation of animals with X or Y-ray doses in the range of 500 rads produces characteristic changes in the biodistribution of a subsequently administered injection of ^{67}Ga-citrate. These changes include reduced soft tissue and blood levels with increased bone concentrations[492,493,494]. Tumor uptake was also reduced but not when local irradiation was applied[493]. In addition, whole body retention was reduced due to increased urinary excretion[492,493]. These effects are likely related to the observed decreases in unsaturated iron binding capacity and increased serum iron levels[493] and this hyperferremic state alters ^{67}Ga-citrate serum binding and subsequent biodistribution. Changes induced by diagnostic chest X-rays cannot be detected by subsequent ^{67}Ga-citrate scans[495].

2) Drugs

a) Drug-induced pathological changes :

A large number of drugs have been shown to be capable of producing altered ^{67}Ga uptake in specific areas due to localized structural changes. The ability of bleomycin[496], cyclophosphamide[497] and busulphan[498] to cause pulmonary interstitial fibrosis and inflammation as well as oily contrast media[499] and BCG (Bacillus Calmette-Guerin)[500] to induce pneumonitis has been found to result in diffuse accumulation of the tracer in the chest region. The lymphoid alterations which may be associated with phenytoin therapy can produce a lymphoma-like ^{67}Ga-citrate biodistribution[501]. The deposition in pseudomembraneous colitis associated mainly with clindamycin

therapy has also been reported[502]. Gynecomastia caused by diethylstilbestrol therapy has also shown ^{67}Ga accumulation[503]. Corticosteroids, which presumably improve the capillary integrity of intracerebral tumors and thus reduce local edema[2], have produced reduced tracer uptake in such areas[504].

b) Drug-induced physiological changes:

The use of methotrexate[506], acetylphenylhydrazine[492], vincristine[493], mechlorethamine[493] and gallium nitrate[506] have been to produce reduced ^{67}Ga serum protein binding[492,493], increased excretion[492,504,505] and a general reduction in soft tissue uptake with increased bone deposition[505,506]. These effects are related to their indirect or direct consequences on the ability of serum transferrin to bind vascular ^{67}Ga-citrate.

The observed increase of thymic uptake in children associated with both antineoplastic[507] and antibiotic[508] chemotherapy may be more related to a diseased induced stress rather than drug induced changes[507].

Antiemetic drugs, such as the phenothiazines, may induce prolactinemia[509], galactorrhea[503] and a resultant breast uptake of ^{67}Ga-citrate. The toxic capabilities of specific antibiotic drugs to induce mild to moderate interstitial nephritis has been shown to cause no change in renal ^{67}Ga deposition[510] but increased kidney uptake has been observed clinically[511].

c) Attempts at pharmacologic enhancement:

Metal Complexes: ^{67}Ga-citrate biodistribution and kinetics are greatly affected by the concurrent use of one of several iron complexes. Studies with mice, rats and rabbits involving either

ferric citrate, ferric sorbitol citrate (Jectofer®) or iron dextran (Imferon®) treatments showed increased ^{67}Ga urinary and fecal excretion, reduced blood levels and reduced uptake in other tissues[390,512-517] and abscesses[517,518] but increased bone concentrations[390,512-517]. Tumor accumulation was only slightly depressed resulting in increased target to background ratios[590,512-516]. This tumor enhancement technique has been used clinically[519-521] but only marginally beneficial results have been obtained, generally in the area of a shortened injection to scanning interval and a reduction of patient radiation dose.

The reported effects of stable gallium citrate administration close to tracer ^{67}Ga-citrate dosing are a general reduction in blood and soft tissue concentrations with a simultaneous increase in excretion and bone uptake[390,522,523]. Tumor levels are also generally reduced, although some enhancement of tumor-to-blood ratios is possible[390,522].

The administration of scandium citrate (0.5 mg/kg) shortly before, with or shortly after ^{67}Ga-citrate results in preferential deposition of ^{67}Ga in bone with reduced soft tissue and blood levels and enhanced excretion[1,390,391,518]. The tracer uptake in abscesses was reduced[518], while depending on species, tumor type and time of analysis, tumor accumulation may be increased[391], unchanged[390,391] or decreased[390]. There is no apparent uptake of radioscandium-46 citrate in tumors at either tracer or carrier levels[391].

Other metal complexes: Other metal citrates have also been shown to produce changes in ^{67}Ga-citrate biodistribution. Indium citrate decreased blood, soft tissues and bone concentrations,

lanthanum citrate decreased blood, liver and bone levels, while magnesium citrate reduced blood and muscle levels but increased bone uptake, and calcium citrate reduced blood and enhanced bone deposition[524].

In general, these observations on the effects of metal complexes on ^{67}Ga-citrate biodistribution are consistent with the theory of displacement or inhibition of ^{67}Ga binding to serum and tissue binding sites by ions which share specific or nonspecific similarities with ^{67}Ga metabolism.

Chelating agents: Deferoxamine mesylate (Desferal®) is a bacterial siderophore which possesses a high affinity for ferric iron and is used clinically to treat iron overload. When administered intramuscularly to animals at various times after ^{67}Ga-citrate, it has been shown to increase ^{67}Ga blood clearance, enhance urinary excretion and reduce tissue uptake. Tumor levels are less affected so that increased tumor to background ratio have been observed in animals[516,525-527]. The appearance of patient scans after deferoxamine treatment were reportedly not improved[528].

A variety of other chelators have been tested in animals with similar results (EDTA, DTPA, DMSA, NTA, cysteine methyl ester, BAL, α α-mercaptopropionylglycine, and α-phthalein complexone[529,430]. D-penicillamine administration produced tumor to blood ratios at 3 hours which were equivalent to those at 24 hours and may be of value in reducing the injection to scanning time. Synthetic agents such as LICAM-C have the capability of removing ^{67}Ga from transferrin and thus the effect of increasing blood clearance may be less dose schedule dependent[531].

The ability of these agents to effectively chelate free ^{67}Ga and thus promote its removal from the blood in a non-protein bound form as well as hindering to some degree the tissue uptake results in an overall increase in tissue to blood ratios.

3) Invasive Procedures

Various simple invasive techniques may result in specific changes in ^{67}Ga uptake, primarily via the induction of inflammatory or infectious foci. Thus, sites of intramuscular drug injections[532], subcutaneous bacterial injections[533] or partially extravascular calcium gluconate intravenous injections[534], as well as surgically created scars[535] and starch peritonitis[536] have been shown to cause local ^{67}Ga accumulation.

Patients on hemodialysis have shown no significant filter retention nor unusual localization of the tracer[537].

VII. Mechanism of Uptake

A) Site Specific Changes

1) Tumors

The wide variation in ^{67}Ga uptake by tumors and normal tissues has been postulated to be related to their rates of cellular proliferation. The viable or actively growing portion of tumor masses have consistently shown the greater ^{67}Ga accumulation compared to necrotic areas[538,539]. Blood clearance in animals possessing fast growing tumors is greater than in those which reproduce at a slower rate[540] and specific mouse tumors have shown a significant correlation between ^{67}Ga uptake and the rate of DNA synthesis[541,542] or rate of growth[543,546]. Hyperactive bone marrow is much more ^{67}Ga avid than normal or depressed marrow[544]. However, studies with cultured Hela cells[541], human lymphocytes[545,546], regenerating rat liver[546], embryonic mouse cells[478], and various rat tumors have shown no correlation between rate of growth and ^{67}Ga incorporation. Thus, in general, the exact membrane or metabolic events which are responsible for ^{67}Ga uptake appear not to be directly related to the degree of cellular proliferation.

Although one may intuitively expect all tumors to be more adequately supplied by blood than normal tissues, this is definitely not the case[548]. The relationship between accumulation of ^{67}Ga and tumor vascularity has been found to be positive, negative or non-existant depending upon the tumor type[549]. Thus, although some blood flow is necessary for tracer delivery, it cannot account for the observed degree of ^{67}Ga uptake in all cases. Changes in vascular permeability in specific areas induced by histamine injection has been

shown to cause local ^{67}Ga accumulation[550], and the presence of more 'leaky' blood vessels in the tumor area may contribute to tracer localization.

The tumor cell membrane possesses different physical properties and functional capacities compared to normal cells. Thus, the suggestion of a hyperpermeable or specifically permeable tumor membrane for ^{67}Ga has been proposed[477,550], but not experimentally substantiated. A relationship between the amount of negative membrane charge of tumor cells and the degree of ^{67}Ga uptake in culture has been reported[543] and is supported by some isolated artifical membrane data[551].

Tumor cells have been found to exist in a more acidic extracellular fluid compared to the normal cellular environment, a fact related to their metabolic status[552]. This feature has been implicated in the tumor localizing ability of ^{67}Ga-citrate, and is supported by some chemical and biological data. ^{67}Ga-citrate has a tendency to adsorb to glass and cellular surfaces which is maximum at pH 4-6. It has also been reported that a large portion of ^{67}Ga-citrate can be precipitated from solution in this range and that the formation of particles are involved in the adsorption and possible internalization of the tracer[553]. This form may be the polymeric complex of ^{67}Ga-citrate or the insoluble trihydroxide.

The dissociation of ^{67}Ga from transferrin has been shown to be greater at reduced pH values[552,554,555] and tumor cells <u>in vivo</u> and <u>in vitro</u> at an induced lower pH have shown greater ^{67}Ga uptake than normal tumor cells, in the absence of any demonstratable membrane changes[552,555,556]. Thus, upon transport to the tumor site, a

decreased local pH may initiate the removal of ^{67}Ga from the carrier protein and the resulting unbound form (possibly a neutral species) may be readily able to diffuse or be carried into the cell. It may be further suggested that once inside the cell the intracellular pH change may be responsible for the metabolic trapping of the tracer and its uptake into specific organelles[557], and thus be responsible for an increase in tumor residence time[558].

2. Inflammatory and Infectious Foci

^{67}Ga-citrate may accumulate at abscess sites by several possible mechanisms involving specific cellular accumulation and/or enhanced local capillary permeability.

^{67}Ga has been shown to be capable of labeling leukocytes *in vivo* and *in vitro*[559] and thus may be able to indirectly infiltrate the lesion via the circulating white blood cells[518,560-562]. However, even in patients with agranulocytosis, some ^{67}Ga uptake into abscesses has been shown to occur[563].

The content of infectious abscesses will invariably contain bacterial cells. Direct ^{67}Ga uptake by these cells has been demonstrated[564] and may involve siderphore production[565]. The presence of polymorphonuclear cells, macrophages and their secretions and breakdown products may also be involved since sterile abscesses accumulate the tracer as well[566]. Hypoxic and non-viable leukocytes take up more ^{67}Ga compared with normal leukocytes[567-569] and the presence of lactoferrin in or secreted by polymorphonuclear leukocytes may irreversibly bind ^{67}Ga at this site[470,471].

It has also been suggested that changes in vascular

permeability play a part in this type of ^{67}Ga localization. Numerous non-specific minor injuries and inflammatory lesions have been shown to take up ^{67}Ga in parallel to ^{99m}Tc-DTPA[570], indicating the size of the extravascular space induced by altered capillary permeability is involved[550,570]. The abscess uptake of ^{67}Ga bound to transferrin suggests vascular changes permit total complex diffuse into these sites[571]. In all likelihood, all of these mechanisms are involved to some degree in the deposition of ^{67}Ga at sites of infection and inflammation.

B) Metal Ion Metabolism

1) Alkaline Earth Metabolism

In general, calcium and magnesium have been found in relatively high concentrations in tumors where they are involved in growth regulation, and in inflammatory foci where they participate in local calcification[572]. Tumors apparently show an increased Ca(II) influx and the ability of ^{67}Ga(III) to follow this route may explain its tumor localizing properties[573]. Many macromolecules which bind calcium and magnesium have also been shown to bind ^{67}Ga, and a competitive process apparently exists[574]. Thus, lipoproteins, nucleoproteins, ATPases, and phospholipids which are intracellular binders of calcium showed some affinity for ^{67}Ga[574-577] and tissues such as liver, spleen and bone which are high in calcium also show high ^{67}Ga uptake[575]. Studies with carrier magnesium and calcium citrate have shown reduced ^{67}Ga uptake in specific soft tissues and increased bone deposition[524]. The ionic displacement theory sums up this proposed mechanism by assuming $^{67}Ga^{+3}$ is capable of replacing

alkaline earth cations in intracellular macromolecules of specific tissues and tumors.

2) Iron Metabolism

The biological behavior of ^{67}Ga-citrate has several features in common with iron complexes and for this reason the mechanism of ^{67}Ga tissue and tumor accumulation may be based on iron metabolism. Numerous studies, in vivo and in vitro, have demonstrated the ability of ^{67}Ga-citrate to bind to animal or human transferrin[386,387,578], the iron carrier protein. The ability of iron and ^{67}Ga to compete for available transferrin sites provides a simple basis for the interpretation of the many physiologic alterations in ^{67}Ga-citrate biodistribution. Thus, the administration of other transferrin bound cations, specific cation chelators or the indirect or direct induction of anemia or hyperferremia by iatrogenic methods subsequently produces changes in the degree of transferrin saturation and the translocation of ^{67}Ga. Factors which generate more unbound ^{67}Ga in the circulation generally result in increased excretion and bone deposition with a reduction of soft tissue uptake. It is reasoned that the nonprotein bound form is readily extracted by the kidney and excreted via the urine and increased bone localization is due to ionic adsorption or displacement of the free form. Soft tissue uptake of ^{67}Ga is primarily from the protein bound pool and thus is reduced when less ^{67}Ga-transferrin is formed. Many cell culture studies have verified the role of transferrin to enhance ^{67}Ga cellular accumulation[579-582]. However, an alternate explanation for the reduced soft tissue uptake in vivo as a result of reduced ^{67}Ga protein binding may be the simple

competition between excretion and high capacity bone uptake with soft tissue extraction.

The protein mediated uptake may take place via either vascular or extracellular transferrin, the latter of which may be more important for tumors. In certain cases where iron loading of the plasma causes the expected reduction in soft tissue uptake, tumor accumulation was not affected[583-585]. In these cases, extravascular transferrin, which is not readily saturated by plasma iron, would maintain ^{67}Ga tumor accumulation from the unbound vascular pool[584]. This type of suggestion may explain both the transferrin enhancement of ^{67}Ga uptake in cell cultures as well as these in vivo observations.

The similar kinetics of ^{67}Ga-transferrin and ^{59}Fe-transferrin in cell cultures have been cited as proof that both species are bound to identifiable transferrin receptors on the cell surface[586,587]. The exact mechanism whereby the ^{67}Ga actually enters the cell may also parallel iron uptake. Some evidence for both complexes favoured the role of adsorptive endocytosis of the total complex[488-591] while other data suggests the protein complex is dissociated at the receptor site with only the metal ion passing into the cell[592-594]. The eventual fate of the tracer may also lie with iron-associated macromolecules such as transferrin, lactoferrin or ferritin, which may act as intralysosomal or cytosolic acceptors[381,423,425,427,430].

However, some studies have shown that transferrin inhibits the uptake of ^{67}Ga[595,596] and although transferrin receptors are more abundant on certain neoplastic cells[128], it has been found that there is no correlation between ^{67}Ga or transferrin uptake and the number of transferrin receptor positive cells[582]. Thus, a transferrin and its

receptor dependent mechanism as well as a transferrin independent system may be in operation.

MATERIALS AND METHODS

I. Preparation of Unlabeled cis-Platinum

A. Synthesis

The synthesis of cis-platinum was accomplished by the initial preparation of an appropriate precursor from available starting materials. Two methods were evaluated for the conversion of this precursor into cis-platinum. All chemicals used in the synthesis were reagent grade unless purification methods are stated and were obtained from local commercial sources. The reactions were generally carried out at the 100 μmolar level and all quantities stated are based on this scale.

1. Synthesis of K_2PtCl_4

The available starting material, $H_2PtCl_6.6H_2O$ (hexachloroplatinic acid hexahydrate) was converted to the tetrachloroplatinite salt by a hydrazine reduction. A weighed aliquot of $H_2PtCl_6.6H_2O$ (51.8 mg) was placed in a 50 ml erlenmeyer flask and dissolved by dropwise addition of 1.0 N HCl. To this solution 14.9 mg of KCl (potassium chloride) was added and evaporated to dryness with low heat, leaving solid K_2PtCl_6 (potassium hexachloroplatinate). Then, 1.0 ml of a freshly prepared 0.1 M aqueous solution of $N_2H_4.2HCl$ (hydrazine dihydrochloride) was added and swirled with the solid. The reaction flask was suspended in a heated water bath (65°C) and stirred for 1 1/2 hours at which time almost all the K_2PtCl_6 had dissolved in the reducing solution forming K_2PtCl_4 (potassium tetrachloroplatinite).

2. Synthesis of cis-Platinum from K_2PtCl_4

a) Direct method: The conversion of K_2PtCl_4 to cis-platinum can be accomplished by the addition of specific concentrations of

ammoniating agents to the tetrachloroplatinite solution as described by Kukushkin[598]. The K_2PtCl_4 was purified by recrystallization from 0.1 N HCl and a 100 μmolar (0.415 mg/ml) solution in double distilled water was prepared. Then, 2.1 μl of a solution containing 400 mg NH_4Cl (ammonium chloride) and 57.6 mg (0.20 ml of a 28.8% solution) of NH_4OH (ammonium hydroxide) per ml was added to 1.0 ml of the K_2PtCl_4 solution and allowed to react at room temperature for 4 hours. The solid products were separated by centrifugation and purified.

b) Indirect method: In the preparation of cis-platinum from K_2PtCl_4 by the indirect method, several sequential reaction steps are carried out, each with a distinct intermediate product, as shown on Figure 5. The method is basically that described by Dhara[599]; Wolf[39]; and Hoeschele[30,600,601].

The K_2PtCl_4 solution [from (a)] was cooled and decanted into a 5 ml Pyrex beaker, and neutralized by the dropwise addition of 0.1-3.0 M NH_4OH to a pH range of 6.5-6.8. Upon the addition of 66.4 mg of KI (potassium iodide), a dark solution containing K_2PtI_4 (potassium tetraiodoplatinite) formed and was stirred for 3 minutes. Then, 1.0 ml of a 0.2 M NH_4OH solution was added and stirring continued for an additional 5 minutes. The precipitate formed during this period was transferred with hot water to a 15 ml Pyrex centrifuge tube, and collected with repeated hot water washes followed by centrifugation. Further washing in this manner with ice cold ethanol and then ice cold ether and subsequent air drying resulted in the collection of a brownish solid of cis-$Pt(NH_3)_2I_2$ [(cis-diamminediiodo-platinum (II)]. A 1.0 ml aliquot of a freshly prepared 34 mg/ml aqueous solution of $AgNO_3$ (silver nitrate) was added to the cis-$Pt(NH_3)I_2$ and vortexed for

3 minutes. After centrifugation the supernatant was carefully removed and placed in a second 15 ml Pyrex centrifuge tube. The AgI (silver iodide) precipitate in the first tube was repeatedly washed with small amounts of hot water until about 5 ml of supernatant containing cis-$[Pt(NH_3)_2(H_2O)_2](NO_3)_2$ [cis-diamminediaquoplatinum(II)nitrate] was collected. To the supernatant, 16.4 mg of KCl (potassium chloride) was added and a white flocculent precipitate of AgCl (silver chloride) formed. Recovery of this supernatant into a 50 ml erlenmeyer flask was followed by several water washings until a final volume of 2-5 ml had been collected. This solution containing the final product was carefully boiled on a hot plate until the volume had been reduced to less than 1 ml at which point the flask was placed in an ice bath. After 1-2 hours, the crystallized product was recovered.

B. Purification and Analysis of Unlabeled cis-Platinum

Cis-platinum was purified by recrystallization from an organic/aqueous solvent[600]. The crude cis-platinum products of the synthetic procedures were dissolved in DMF (dimethylformamide) to a concentration of 10-20 mg/ml in a 15 ml Pyrex centrifuge tube. This solution was centrifuged to remove suspended or insoluble impurities and the soluble phase transferred to a second centrifuge tube. Two volumes of 0.1 N HCl were mixed into the DMF solution and the tube was kept in an ice bath until the purified cis-platinum recrystallized from this solution, usually within 2 hours. The compound was examined visually as a rough qualitative guide to compound purity, with additional characterization by chemical, chromatographic and spectrometric analyses, as described below.

1. Chemical Test

A chemical test for cis-platinum identification based on its specific reactivity with thiosulphate was performed[602]. About 5 mg of cis-platinum was placed in a small glass test tube with 500 µl (~ 8 mg) of $Na_2S_2O_3$ (sodium thiosulphate) solution and warmed to dissolution over a low flame. A small amount of potash was added and the mixture swirled for several minutes. After cooling and the addition of 2 ml of 95% ethyl alcohol, the tube was vigorously shaken. The reaction products could then be identified and related to the original nature of the platinum reactants.

2. Chromatographic Tests

Chromatographic analysis of cis-platinum products was performed using two different methods. The first consisted of Whatman #1 filter paper strips and a solvent system of 0.2 M glycine in distilled water[28]. The strips (15 x 2 cm) were spotted 2 cm from the bottom and developed to a height of at least 10 cm in a Gelman ITLC tank or a Brinkman chromatography tank. The second system utilized Whatman MK6F (40A° silica gel with fluorescent indicator) analytical TLC precoated plates as the stationary phase and developed in an acetone:water (9:1) solvent[34]. The plates were spotted 1 cm from the bottom and developed to a height of 5-6 cm. The analyte compounds were dissolved in 0.1 N HCl and 1-8 µg were spotted per run. After development, the chromatograms were visualized by iodine vapour[603] (and U.V. visualization for the fluorescent plates). Compound identification was cross-checked with recrystallized reference materials of cis-platinum, trans-platinum, K_2PtCl_4 and H_2PtCl_6. (Mathey-Johnson

Co. or Fischer Scientific Ltd.)

3. Spectrophotometric Test

Purified cis-platinum was subjected to ultraviolet absorption analysis using a Unicam U.V. spectrophotometer. The compound was dissolved in 0.1 N HCl and scanned from 225 to ~450 nm in the automatic wavelength scan mode. The absorption spectra were recorded on a Unicam AR 25 Linear recorder.

II. Detection of Platinum

A. Use of Labeled cis-Platinum

1. Preparation

Two methods were evaluated for the preparation of the labeled compound. The first involved irradiation of an appropriate precursor followed by synthetic procedures and the second relied on direct thermal neutron bombardment of cis-platinum itself.

a) Synthetic method: A given quantity (5-20 mg) of K_2PtCl_4 was subjected to a thermal neutron flux of 1×10^{12} $n.cm^{-2}$ sec^{-1} for 4 hours or 2.5×10^{11} $n.cm^{-2}.sec^{-1}$ for 16 hours at the University of Alberta Slowpoke II Reactor Facility. After 4 hours of cooling a 100 μmolar solution of radioactive material was prepared in 1.0 N HCl. Next, 2.0 ml of DBS (dibutyl sulphide) or MIBK (methyl isobutyl ketone) was added to 2.0 ml of the radioactive K_2PtCl_4 solution. The mixture was vortexed for several minutes and allowed to equilibrate for 15 minutes after which the organic phase was withdrawn and discarded. This extraction procedure was repeated to ensure the complete removal of all ^{199}Au formed as a result of the irradiation

process. Two 1 ml portions of the remaining aqueous phase was used as the starting material for the conversion to labeled cis-platinum via the indirect method previously described.

b) Direct activation: Purified cis-platinum (10-20 mg) was irradiated in a thermal neutron flux of approximately 5×10^{11} $n.cm^{-2}.sec^{-1}$ for 2 hours at the Slowpoke II Reactor Facility. This irradiation was carried out using the single outer capsule site of the reactor. The irradiated compound was allowed to cool for 4 hours, recrystallized from DMF:2 HCl and redissolved in 0.01 N HCl. This solution was extracted twice with an equal volume of DBS to remove ^{199}Au activity.

2. Purification and Analysis of Labeled cis-Platinum

Labeled cis-platinum from the synthetic method was purified by recrystallization by a method identical to that of the unlabeled material. The labeled compound prepared by direct activation was recrystallized similarly except that the radiogold extraction step was performed after purification.

a) Radiochromatographic tests: Radiochromatographic analysis was performed using the labeled compound in both systems utilized for the unlabeled material. Detection of chromatographic spots was by iodine vapour visualization with activity analysis provided by whole chromatogram counting under a Berthold Linear TLC Analyzer, or by sectional counting of the paper strips in an automatic multisample gamma well counter.

b) Spectrophotometric test: Several purified samples of labeled cis-platinum were subjected to U.V. analysis similar to the

unlabeled compound.

3. Counting of Labeled cis-Platinum

Radiolabeled cis-platinum was detected by either solid scintillation or liquid scintillation type detectors and their associated electronics.

a) Gamma counting: The Tracor Gamma 2200 multisample NaI(Tl) 3" x 3" well counter was used for counting cis-platinum samples. This unit was coupled to a Tracor model 1710 multichannel analyzer system which accessed a mini-computer, decwriter, disc storage and spectral printout facilities.

b) Liquid scintillation counting: Cis-platinum was dissolved in DMF and was then easily incorporated into a standard fluor solution (4 g PPO, 50 mg POPOP in scintillation grade toluene). These samples were counted in a Beckman LS 9000 liquid scintillation spectrometer using a window from Channel 400-900. Total spectra printout capabilities were available from magnetic tape stored data.

B. Atomic Absorption Spectrometry

Flameless atomic absorption analysis of platinum was performed using a Perkin-Elmer Model 305B Atomic Absorption Spectrophotometer equipped with a Deuterium Arc Background Corrector and a HGA-2000 Graphite Furnace system. The atomic platinum signal (2659.5 nm) was generated within the instrument by a Westinghouse hollow cathode device (type WL 36051) operated at 15 mA. Analysis was performed using 50 µl injection volumes, a slit width of 1.0 mm (0.7 nm spectral band width) and automatic recorder control and purge gas (N_2 at 1.0

psi) interrupt modes. The graphite tube operating conditions were selected for platinum determination as described by the instrument manufacturer and consisted of a dry cycle (30 seconds at 125°C), a char cycle (30 seconds at 1600°C) and an atomize cycle (10 seconds at 2700°C). Individual graphite tubes have a finite lifetime due to deterioration of heat transfer capabilities causing loss of efficiency, and were routinely replaced after 80-120 determinations. Following each analysis, the tubes were subjected to a 15-20 second maximum temperature cycle for removal of residual material. Absorption signals were recorded as peaks on a Beckman 10" chart recorder with peak heights used as a measure of platinum concentration. Standards were prepared in the identical aqueous systems as the analyte solutions and used to calibrate the procedure. Each standard was analyzed at least six times while the test solution determinations were performed at least twice. The calibration curve was checked periodically to assure accuracy and reproducability.

C. Neutron Activation Analysis

A technique for the determination of platinum (and gold) in biological materials by neutron activation analysis was developed. Several aspects of the procedure were investigated in order to optimize conditions for the assay.

Some common digestion mixtures that utilize acidic oxidation for tissue solubilization were subjectively evaluated. These consisted of combinations of concentrated (conc.) HNO_3, conc. $HClO_4$ or 35% H_2O_2 with conc. H_2SO_4. Assessment was based on whether solubilization could be accomplished in a reasonable time period; the heating

requirements; the persistance of residual oxidant at completion; and the quantitative extent of radiogold loss. The determination of this last factor was performed using 4.0 ml of a 1:1 mixture of conc. H_2SO_4 and conc. HNO_3. The sample consisted of ^{195}Au (New England Nuclear) bound to a freeze dried serum matrix packaged in a gelatin capsule (Eli Lilly and Co., size 1-3). The samples were counted (Beckman Gamma 8000) in individual test tubes (16 x 1 1/2 cm) prior to the addition of digestant and 100 µg carrier gold. The tubes were placed in temperature-controlled oil baths and at specified times were removed, cooled, centrifuged to collect any condensate and recounted. This procedure was verified in several cases by collection of the digestion vapours via a condenser apparatus with recovery and counting of the distillate.

The effect of the addition of NaBr (sodium bromide) to radiogold and radiobromine extraction was measured by adding 0 mg (control), 200 mg or 500 mg NaBr to 2.0 ml conc. H_2SO_4 containing ~10,000 cpm ^{195}Au and a similar quantity of $NH_4{}^{82}Br$, (prepared on site by thermal neutron irradiation of cold compound) followed by the optimized digestion and extraction procedures. A correction factor for the spillover of ^{82}Br into the ^{195}Au channel was established during initial studies.

The effect of the possible second order interferences via $^{46}Ca(n,\gamma)^{47}Ca \rightarrow {}^{47}Sc$ (159 KeV gamma) or $^{197}Au(n,\gamma)^{198}Au(n,\gamma)^{199}Au$ were assessed by spiking samples with 100 mg of calcium carbonate solution or 2 mg gold wire, followed by the irradiation, digestion and extraction procedure.

The optimized digestion and extraction procedure, based on the

results of these studies is given using human plasma as an example. Human plasma samples were lyophilized and weighed aliquots (50-100 mg) placed in envelopes constructed from low ash filter paper. Samples were loaded into standard polyethylene vials and irradiated in a neutron flux of 1×10^{12} $n.cm^{-2}.sec^{-1}$ for 4 hours at the University of Alberta Slowpoke II Reactor Facility. The activated samples were allowed to cool for 24-48 hours prior to the initiation of radiochemical procedures.

Each sample (plus its paper envelope) was digested in a test tube (16 x 1 1/2 cm) containing 2.0 ml conc. H_2SO_4, 200 mg NaBr, 25 µl ^{195}Au solution (≈ 0.5 µCi/ml) and 100 µg gold carrier (1 mg/ml in 10% HCl). Tubes were placed in an oil bath at 105°C and gradually increasing volumes (50-500 µl) of fresh H_2O_2 (30-35%) were added, at several minute intervals to allow the reaction to proceed, until a total of 1.5 to 2.0 ml had been used per sample. More or less can be used depending upon the nature of the sample to be solubilized. Several aliquots of concentrated HCl were slowly added (total 1.0 ml) and heating continued for 15-20 minutes until the digest were clear and no gaseous oxygen was generated within the digest solution. The digests were cooled and centrifuged to collect residual condensate from the sides of the tubes. A known volume (2-3 ml) of dibutyl sulphide (1.0 M in toluene) was added and the mixture vigorously vortexed for 10 minutes to ensure thorough contact of the phases. Sample tubes were then centrifuged and permitted to equilibrate for a minimum of 10 minutes, after which time the organic phase was withdrawn and a measured aliquot taken for counting.

Standard curves were generated by irradiating plasma samples

spiked with known quantities of platinum followed by this procedure. The extracts were counted in a cave with 10 cm lead walls using a WIN-15 coaxial Ge(Li) spectrometer coupled to a ND660 multi-channel analyzer system.

III. Detection of Radiogallium

^{67}Ga was the only radioisotope of gallium used in this project. Its gamma emissions were counted using a variety of detection equipment and associated electronics:

1. Picker Autowell II
2. Tracor Gamma Trac 2200
3. Beckman gamma 8000
4. Nuclear Chicago Pho/Gamma IV
5. General Electric 400 Maxicamera
6. Shielded NaI(Tl) detector with associated modular high voltage supply unit, discriminator unit, and scalar

IV. Protein Binding Analysis

All *in vitro* transferrin binding studies were conducted in a solution of buffered saline. This consisted of 0.04 M TRIS [tris(hydroxymethyl)aminomethane] adjusted to pH 7.4 by HCl. Chloride ion concentration was brought to 0.10 M by the addition of NaCl.

A. Ultrafiltration

Centriflo membrane cones (Amicon Inc. type CF25) with a molecular weight cut-off of 25,000 atomic mass units were used for ultrafiltration evaluation. The cones were pre-soaked in double

distilled water for at least one hour prior to use to remove the glycerol residue as recommended by manufacturer. The cones were then centrifuged at 1500 rpm (< 500 g) for 5 minutes to remove residual water. The test solution (4-5 ml) was added and the unit centrifuged again. This initial ultrafiltrate (< 200 µl) is discarded. A third centrifugation results in the collection of an additional 40-200 µl of solution and a measured aliquot is taken from this fraction to calculate the amount of free or unbound activity in the test solution. In some cases, an identical volume was removed from the test solution in the cone which corresponds to the total (free and bound) activity. The fraction of ^{67}Ga bound is calculated by subtracting the counts of the ultrafiltrate from the total counts and dividing this value by the total counts (identical counting times and solution volumes).

B. Dialysis

The dialysis technique for the in vitro transferrin studies was performed using a self-designed unit constructed from available laboratory materials. The dialysis tubing (Fischer Scientific Co.) was knotted at one end with additional leakage protection provided by a cotton thread tied securely around the distal end of the knot. The other end of the tube was passed through and then folded over the outside of a plastic fitting and held in place by a small elastic band or thread. The fitting was made by cutting a 10 ml syringe barrel 1 1/2 cm from the finger grips and buffing the cut edges so as not to be abrasive to the delicate tubing. The rest of the fitting is shaped to enable the whole unit to fit snugly in a flexible 50 ml polyethylene screw-top centrifuge tube (Falcon; Div. Becton, Dickinson & Co.). The

analyte solution is placed in the dialysis tube and enclosed by the careful insertion of a moistened plunger into the dialysis tube unit. The plunger was shortened to about 2 cm in length for this purpose.

With the addition of dialysing solution to the centrifuge tube which was closed with the screw cap, the system can be readily shaken or rotated for dialysis experiments. Access to the dialysing solution for analysis is easily accomplished by removal of the screw cap and careful removal of the small plunger from the dialysis tube unit allowing the sampling of the dialysate. The dialysis tube unit should not be totally submerged in the dialysing solution to facilitate repeated sampling from inside the dialysis membrane.

The volume of sample accommodated by the dialysis tube was 4-5 ml; whereas 40-45 ml of dialysing solution was placed in the outer centrifuge tube. The percentage of ^{67}Ga activity bound was calculated by subtracting the concentration of activity outside the tube from that inside the tube and dividing by the total of these two values.

Reagents for dialysis experiments consisted of freshly prepared aqueous buffered saline solutions of: K_2PtCl_4 or cis-platinum (2.5×10^{-6} moles/ml); nitrilotriacetic acid (NTA) (2.50×10^{-4} moles/ml); human apotransferrin (Sigma Chemical Co.) (1.25×10^{-8} moles/ml) and ^{67}Ga-citrate (1-100 μCi/10 μl in 0.1 N HCl). The test solutions were prepared by adding 1.0 ml of either of the platinum solutions to 100 μl of NTA solution which had been previously incubated at room temperature for one half an hour with 10 μl of $^{67}GaCl_3$. This was followed by the addition of 1.0 ml of transferrin solution and 2.0 ml buffered saline.

The whole mixture was incubated at 4°C for 24 hours. It was then

placed in a dialysis tube and fitted into a 50 ml plastic centrifuge tube containing 40 ml of buffer. Dialysis units were then allowed to rotate for 24-72 hours with intermittent sampling. Three units per platinum compounds plus three controls of each series were run. The control series consisted of units containing all of the above materials and no transferrin and a second series containing the above materials omitting the addition of platinum compounds. Equilibration was established within 18 hours under these conditions.

The dialysis technique for the ^{67}Ga-citrate/platinum *in vitro* serum binding studies involved enclosing the serum and reagents in the same dialysis membranes, sealed with membrane clamps and dialysing against an exact measured volume of dialysing medium of buffered saline. The percent bound was calculated by sampling the medium and comparing it to a measured aliquot of the activity initially placed in the dialysis tube. Serum samples consisted of 2.8 ml serum (Red Cross, Edmonton), 100 µl of labeled or unlabeled cis-platinum or K_2PtCl_4 (3.33 mM in 0.1 M NaCl), 50 µl 0.1 M $NaHCO_3$ and 50 µl ^{67}Ga-citrate (2-10 µCi). The effect of pre-incubation of the platinum compounds with the serum for 24 hours before addition of ^{67}Ga-citrate was also tested.

Plasma samples obtained from rabbits before and after cis-platinum treatment were also subjected to dialysis for the determination of the degree of protein binding. Samples (400-500 µl) were placed in smaller dialysis tubes (0.5 x 8 cm) sealed with membrane clamps and dialysed against 40.0 ml of buffered saline for 72 hours. Aliquots of the dialysing medium were compared against standard plasma samples for the calculation of percent ^{67}Ga bound

under these conditions.

C. Gel Chromatography

Gel chromatography was performed using a Sephadex G-50 exclusion gel. The column was prepared by warming an appropriate quantity of the gel in distilled water, cooling and pouring into a 18 x 1 cm plastic support tube. The height of the gel was about 15 cm after settling and was equilibrated with buffered saline prior to use. The test solution was applied to the top of the column in a volume not exceeding 0.5 ml and eluted with additional buffer. The eluate flowed via polyethylene tubing in front of a NaI(Tl) radioactivity monitor and subsequently through an ultraviolet detection cell. Output from these detectors (count rate and absorbance at 280 nm, respectively) was recorded on a chart graph display. The final eluate was automatically collected in 1-2 ml fractions (Fracto Mette 200 fraction collector, Buchler Inst. Co.). These fractions were counted by a Gamma Trac 2200 multisample automatic NaI(Tl) detector and results recorded in terms of the percentage of ^{67}Ga activity in each fraction. Additional pre-programmed calculations were then able to analyze the data for the relative percentage of activity in each of the well defined chromatographic peaks. After the decay of ^{67}Ga, these fractions were analyzed for their platinum content by atomic absorption spectrometry.

Test solutions for the gel chromatographic experiments consisted of either cis-platinum (purified by recrystallization from DMF:2HCl) or K_2PtCl_4 (purified by recrystallization from 0.1 N HCl), a specified anion (citrate/bicarbonate or NTA), transferrin and $^{67}GaCl_3$ in an

aqueous buffered solution. Platinum compounds were dissolved in distilled water to a concentration of 1.88 μmoles/ml. Anion solutions of NTA and of citrate/bicarbonate were also prepared, the first containing 3.76 μmoles/ml NTA and the second contained 30 μmoles/ml citric acid and 1.5 mmoles/ml sodium bicarbonate. Two ml of each of the platinum solutions were mixed with two ml of each of the individual anion solutions and the mixtures incubated at 4°C for 24 hours. At that time, eight ml of a 1.25 mg/ml human transferrin (Sigma Chemical Co.) in solution was added. After a further incubation period of 24 hours at 4°C, 10 μl of $^{67}GaCl_3$ solution (in 0.1 N HCl) was added and the mixtures stored at 4°C for at least three days with intermittent agitation until chromatographic separation. Two control solutions, one containing no platinum compounds, the other containing no transferrin were prepared in an analogous manner.

A summary of the incubation conditions for the dialysis and gel chromatography experiments is given in Table 7. A schematic diagram of the various analytical steps for the gel chromatography experiments is shown in Figure 4.

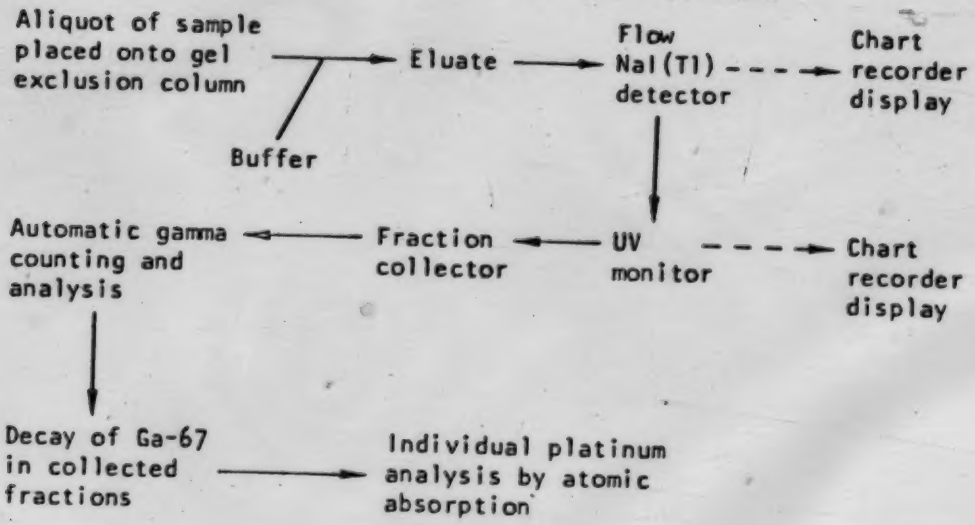


Figure 4. Sequence of the gel chromatographic separation procedures for the determination of Ga-67 and platinum binding to transferrin in vitro.

Table 7. Summary of incubation conditions for dialysis and gel chromatography studies of ^{67}Ga binding to transferrin *in vitro*.

Reagent	Incubation Mixture Concentration (M)	
	Dialysis	Gel Chromatography
cis-platinum	6.1×10^{-4}	3.1×10^{-4}
K_2PtCl_4	6.1×10^{-4}	3.1×10^{-4}
NTA	6.2×10^{-2}	6.2×10^{-4}
HCO_3/citrate	-	$5.3 \times 10^{-3}/2.5 \times 10^{-1}$
Transferrin	3.0×10^{-6}	1.0×10^{-5}
TRIS buffer	0.04	0.04
Chloride	0.075	0.067
H^+	3.98×10^{-8} (pH = 7.4)	3.98×10^{-8} (pH = 7.4)
$^{67}GaCl_3$	~ 50 μCi	~ 50 μCi
Platinum: transferrin ratio	200:1	30:1
Incubation duration	24 hours	> 72 hours

V. Animal Studies

A. Rodent Experiments

The effects of a specific cis-platinum treatment schedule on ^{67}Ga distribution at various times after tracer administration was studied in healthy male adult (300 - 400 g) Sprague Dawley rats. They were maintained on rodent chow (Wayne Laboratory Animal Diet) and fresh water *ad libitum*. The rats were divided into a treatment group and a control group. The treatment group received 0.35 mg/kg of purified

cis-platinum in normal saline (1 mg/ml) by intraperitoneal injection for five consecutive days while the control group received an identical injection schedule with normal saline. Immediately after these injections on the fifth day, both groups were given a 10 - 50 μCi intraperitoneal or intravenous injection of ^{67}Ga-citrate in normal saline (~ 0.2 ml). Animals sacrificed at periods shorter than 12 hours were given intravenous injections of ^{67}Ga-citrate, while those sacrificed at longer periods were injected intraperitoneally with the tracer since at these later times there is no difference in the observed tissue distribution based on the route of injection[394]. At specific times thereafter (3, 6, 12, 24, 48, 72, and 168 hours), three animals from each group were sacrificed for tissue distribution analysis.

Studies in mice were utilized to simultaneously assess the possible effects of a medium level acute dose of cis-platinum and successive long term accumulated doses of cis-platinum on the short term distribution of ^{67}Ga-citrate. CBA/CAJ mice (18-25 g) were divided into five groups of 6 mice each. The first group served as the control group and received 0.5 - 2 μCi ^{67}Ga-citrate in normal saline by intravenous injection, and were sacrificed 3 hours later. The second group received 0.5 - 2 μCi of ^{67}Ga-citrate and 5 mg/kg cis-platinum co-administered by intravenous injection, and were sacrificed 3 hrs later. The remaining groups were given 5 mg/kg cis-platinum by intraperitoneal injection every second day with the last dose co-administered with ^{67}Ga-citrate by intravenous injection to give successively higher cumulative doses, and similarly sacrificed at 3 hours after the last dose. In addition, radiolabeled

cis-platinum was administered to a separate group of mice for the determination of its distribution at 3 hours after intravenous injection.

A second series of mouse (CBA/CAJ with transplanted RI lymphoma tumors) experiments involved the use of other chemotherapeutic agents in a particular regimen to evaluate the short and long term effects on ^{67}Ga-citrate distribution after an acute dose. Three regimens were evaluated against saline controls by animal sacrifice at 3 and 48 hours after intravenous injection of drugs and 0.5 - 2 μCi of ^{67}Ga-citrate. The first group received 0.1 mg/kg vinblastine (Velbe®), 40 mg/m^2 doxorubicin (Adriamycin®) and 30 units per animal of bleomycin (Blenoxane®) plus ^{67}Ga-citrate, the second group received these agents plus 20 mg/m^2 cis-platinum, and the third group received only 20 mg/m^2 cis-platinum and ^{67}Ga-citrate.

Rodents were sacrificed by chloroform or ether inhalation. Immediately after death, the chest was opened and a blood sample was obtained from the heart. Whole organs were removed from the animals and blotted to remove residual blood. Stomach and large intestine contents were also removed. Tissues were subsequently weighed and counted to less than 0.5% error in an appropriate gamma counter.

B. Rabbit Experiments

The basic study protocol was designed to compare the biological behavior of ^{67}Ga-citrate in the same rabbits before (control) and after treatment with cis-platinum, or where sacrifice was necessary, a comparison between two matched sets of animals. Various physiological parameters were also assessed as the possible determinants of any

cis-platinum induced alterations of ^{67}Ga-citrate disposition.

Twelve healthy adult New Zealand White rabbits of both sexes, with a weight range of 3-5 kg were used in these studies. The rabbits were housed in individual metal cages equipped with a screened tray to facilitate urine and feces collection. Food (Master Feeds, Maple Leaf Milk Ltd.) and fresh tap water was available ad libitum. Prior to the initiation of any experiments, the animals were allowed to acclimatize for a minimum period of two weeks.

In the control phase of the study, ^{67}Ga-citrate (New England Nuclear) was administered by an intravenous bolus injection via a lateral auricular vein with a dose of 0.05 - 0.9 mCi (1.8 - 33 MBq) in 0.2 - 0.4 ml of normal saline. ^{67}Ga behavior was evaluated for a period of one week, and after a further three weeks (when the same animals were to be used), such that ^{67}Ga activity was negligible, cis-platinum treatment was started. Purified cis-platinum (Johnson, Mathey & Co.) was administered daily as a 0.5 mg/kg intraperitoneal injection in saline (1.0 mg/ml) for five consecutive days. Five days after the completion of cis-platinum treatment, ^{67}Ga-citrate was (re)administered as in the control phase and (re)evaluation of ^{67}Ga behavior initiated for the cis-platinum treated phase.

1. Measurement of ^{67}Ga-citrate Biological Behavior in Rabbits

Several aspects of the biological behavior of ^{67}Ga-citrate were evaluated in the control and cis-platinum treated animals.

^{67}Ga localization: Whole body distribution of ^{67}Ga at 48 hours after injection was determined by sacrifice of a treated and non-treated group of female rabbits. Tissue samples were carefully

removed, rinsed in saline, weighed, counted in a multisample well counter and the results expressed as the percent of the dose per gram of tissue.

Two rabbits were scanned at 12, 24 and 48 hours after ^{67}Ga-citrate injection using a General Electric Maxicamera 400 (Dr. R. Hooper) during both the control and cis-platinum treatment phases.

^{67}Ga elimination: Whole body elimination of ^{67}Ga was measured by counting the animals with reproduceable geometry with both a Searle Pho/Gamma IV gamma camera equipped with a non-collimated detector headpiece, and a shielded 3" x 3" NaI(Tl) crystal and associated counting equipment. The daily radioactivity was measured, corrected for decay and background and compared to the activity recorded at 2 - 10 minutes after ^{67}Ga-citrate injection, which served as the 100% injected dose value for calculation of the percent of the dose remaining each day.

In addition, total urine volume and fecal mass were measured daily in several animals and representative aliquots taken for counting. These were compared to appropriate dilutions of the injected ^{67}Ga dose for the determination of daily urinary and fecal excretions.

^{67}Ga pharmacokinetics: At various times after ^{67}Ga-citrate injection (at least four times during the first hour, five times during the next 23 hours and once a day thereafter) blood was withdrawn without anticoagulant from a vein in the opposite ear from the injection site. The blood was immediately transferred to a tared counting vial and a net weight obtained. The samples were counted along with dose standards, decay and background corrected and

expressed as the percent of the dose per gram of blood. Pharmacokinetic analysis of individual animal data was performed using both AUTOAN and NONLIN computer programs[604,605]. AUTOAN was used to obtain initial estimates of the blood level-time curves with final curve fitting by NONLIN. The number of exponential terms was chosen on the basis of statistically ($p < 0.005$) significant reduction in the sum of the squared deviations, and the randomness of the data points[606].

^{67}Ga protein binding: Serum samples were obtained from rabbits before initiation of cis-platinum treatment and 24 hours after its five day injection schedule was completed. These samples were used to assess its binding capacity for ^{67}Ga-citrate *in vitro*. Serum (500 µl) was incubated with ^{67}Ga-citrate (~ 0.5 µCi) for three hours at room temperature prior to separation.

Serum samples were also obtained from rabbits at 3 and 48 hours after ^{67}Ga-citrate injection in both control and treated animals to assess ^{67}Ga protein binding *in vivo*. Samples were separated by 72 hour dialysis against 0.037 M TRIS/HCl buffer (pH 7.4) and by gel exclusion chromatography using a 20 cm G-50 Sephadex column eluted with the same TRIS buffer as described previously.

Urinary disposition: Urine samples were collected 24 hours after ^{67}Ga-citrate injection and 4 ml portions centrifuged to partition the sediment fraction. Both fractions were counted for ^{67}Ga activity to determine the percentage of ^{67}Ga in the soluble fraction. The pH was determined using a Radiometer PHM 82 standard pH meter. Several urine samples were pH adjusted with HCl or NaOH and the separation process repeated to indicate the effect of pH on the solubility of endogenous

^{67}Ga-citrate.

2. Measurement of Specific Physiological Parameters of Rabbits

Several physiological parameters were also measured as an indication of the toxicity of cis-platinum and as possible indirect causes of alterations of ^{67}Ga-citrate biological behavior.

Hematological parameters: Serum creatinine, hematocrit, total iron and total iron binding capacity (TIBC) were measured at various times in control and cis-platinum treated phases. Serum creatinine analysis was performed by the University of Alberta Surgical Medical Research Institute (M. McCubbin) and total iron and TIBC by the University of Alberta Hospital (Dr. A. Dinwoodie).

Consumption parameters: Food and water intake was measured on a daily basis, and body weight measured at various times during the control and treatment phases.

Pathological parameters: Kidney slices obtained at 7 and 15 days after cis-platinum treatment were analyzed for morphological changes at the Edmonton General Hospital (Dr. D. Williams).

VI. Statistical Methods

Standard statistical analysis was carried out on all data where the resultant p (probability) values are indicated with asterisks. The analysis requires the mean and corresponding standard deviation (s.d.) values as well as the number of data for each group (n). Some of the calculated statistics resulted in levels of significance much higher than the 99% shown ($p < 0.01$), however, for a more readily appreciated display, usually only the two generally accepted levels

are indicated[607].

Three statistical tests were employed to test the statistical difference between two or more groups of mean values. The paired or unpaired t test were used for all analysed data except the two experiments involving mice. The design of these two studies allowed the data to be analysed by an ANOVA (analysis of variance) with specific comparisons with the control group based on the LSD (least significant difference) test.

RESULTS AND DISCUSSION

I. Preparation of Unlabeled cis-Platinum

Since pure cis-platinum was not available when this project was initiated, it was imperative to synthesize the compound using established methods. Commercially available cis-platinum preparations contain excipients such as mannitol and sodium chloride which make them unsuitable for the conduct of the planned binding studies. In addition, the *in vivo* studies may also be affected by the preparation composition since mannitol complexation and chloride ion concentration must be taken into account. Thus, in order to obtain a chemically pure drug, synthesis and quality control procedures were undertaken.

A. Synthesis

Elemental platinum is the most common target material for platinum radioisotope production and is converted to H_2PtCl_6 by dissolution in aqua regia. The hexachloroplatinate was readily available as the hexahydrate and prospects of a labeled synthesis made it a suitable choice as a starting material. Figure 5 gives the sequence of reactions for the preparation of cis-platinum from $H_2PtCl_6 . 6\ H_2O$.

1. Synthesis of K_2PtCl_4

The acid solution of H_2PtCl_6 is converted to the salt form by the addition of KCl and evaporation to dryness (reaction I). This platinum complex must be reduced to the tetrachloro derivative before ligand exchange takes place. Hydrazine salts[608] or oxalic acid[609] have been employed to reduce Pt(IV) to Pt(II) complexes of this type. Both reductants produce reaction products which can be easily

Rx No.

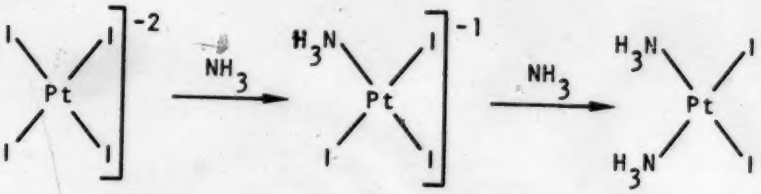
I $H_2PtCl_6 \cdot 6H_2O + 2KCl \longrightarrow K_2PtCl_6 + 2HCl + 6H_2O$

II $K_2PtCl_6 + \frac{1}{2}N_2H_2 \cdot 2HCl \longrightarrow K_2PtCl_4 + \frac{1}{2}N_2 + 3HCl$

III $K_2PtCl_4 + 4KI \longrightarrow K_2PtI_4 + 4KCl$

IV $K_2PtI_4 + 2NH_4OH \longrightarrow cis\text{-}Pt(NH_3)I_2 + 2H_2O + 2KI$

IVa



V $cis\text{-}Pt(NH_3)_2I_2 + 2AgNO_3 + 2H_2O \longrightarrow cis\text{-}[Pt(NH_3)_2(H_2O)_2](NO_3)_2 + 2AgI$

VI $cis\text{-}[Pt(NH_3)_2(H_2O)_2](NO_3)_2 + 2KCl \longrightarrow cis\text{-}Pt(NH_3)_2Cl_2 + 2KNO_3 + 2H_2O$

Figure 5. Reaction sequence for the synthesis of cis-platinum.

separated (N_2 and CO_2 respectively) and those which do not interfere with subsequent reactions. Hydrazine has been utilized for this purpose to a greater extent and is used here. Of the hydrazine compounds available, hydrazine dihydrochloride is best suited since it is a solid and therefore exact amounts can be simply weighed, whereas hydrazine sulphate is poorly water soluble and hydrazine monohydrate is a noxious liquid[610]. Excess of this reducing agent must be especially avoided due to the possible formation of tetravalent or bivalent platinum hydrazine complexes[611] and due to the reduction of some platinum species to elemental platinum, a process which may be pH and concentration dependent[34]. Thus, the stoichiometry is critical for reaction II. The extent of the reaction can be roughly monitored by the dissolution of the yellow K_2PtCl_6 and the formation of a red solution of K_2PtCl_4. The reaction is allowed to proceed at 65°C, although it will also proceed at reduced temperatures. If traces of undissolved K_2PtCl_4 remain or elemental platinum is present, the solution can be filtered or decanted leaving the K_2PtCl_4 solution. Losses due to this reduction step are about 15-20% which are acceptable in terms of final product purity.

2. Synthesis of cis-Platinum from K_2PtCl_4

The conversion of K_2PtCl_4 to cis-$Pt(NH_3)Cl_2$ can be accomplished either directly using buffered ammonia reagents or indirectly via the tetraiodo analogue.

a) Indirect method: The indirect method takes advantage of the greater trans-effect characterizing the tetraiodo intermediate and its synthesis is accomplished by the addition of KI to the K_2PtCl_4

solution. The optimum I^-/Pt for this reaction in terms of the stability of the product and the relative yield has since been found to be six rather than the stoichiometric four[30]. The rate of this reaction can be increased by the application of heat but the product is unstable above 40°C[598]. Thus, the reaction III proceeds to the more stable product and the formation of a characteristic dark solution of PtI_4^{-2}. The trans-effect of the tetraiodo derivative is realized in the next reaction where two of the iodide ligands are replaced by ammonia. The trans-effect can be defined as the effect of a co-ordinated group upon the rate of substitution reactions of ligands opposite to it in a metal complex. The approximate order of decreasing trans-effect for Pt(II) complexes is CO, CN^-, $C_2H_4 > CH_3 > C_6H_5^-$, NO_2^-, I^-, SCN^-, $> Br^-$, $Cl^- > NH_3$, H_2O[612]. Once an ammonia molecule has entered the complex, the next most labile ligand will be one of the iodide ions trans to each other rather than that trans to the ammonia since iodide has a greater trans-effect than ammonia and since the platinum-nitrogen bond is stronger than the platinum-iodide bond. This results in the formation of the cis isomer as shown in IVa. Since this is a kinetic effect, the reaction takes place at a much faster rate than would the corresponding reaction for the tetrachloro derivative. Thus, the addition of the required quantity of NH_4OH to the dark K_2PtI_4 solution results in the rapid formation of cis-$Pt(NH_3)I_2$ which precipitates from solution as a light brown product according to reaction IV. This complex is removed from the solution by centrifugation and washed with water to remove soluble impurities.

The addition of $AgNO_3$ solution to the solid cis-$Pt(NH_3)I_2$

results in the formation of the soluble diaquo complex. This reaction V is driven to this end point due to the formation of the highly insoluble AgI. A 10% excess of $AgNO_3$ is added to ensure complete removal of all iodide while warm water washing of the AgI precipitate prevents the loss of the diaquo product.

Water is one of the best leaving groups for Pt(II) complexes and is easily replaced by chloride, supplied by KCl or HCl, to give the desired product. These reagents are added initially in about 50% of the stoichiometric amount which results in the precipitation of excess Ag^+ as AgCl while allowing the diaquo species to remain in solution[30]. After removing and washing the solid AgCl, a slight excess of KCl or HCl was added to complete the reaction VI. Depending on the volume of the final solution (which depends upon the concentration of reagents and the volume of washings used) it may be necessary to boil the final solution prior to cooling to 0°C to complete the precipitation.

The indirect synthesis from K_2PtCl_4 to cis-$Pt(NH_3)_2Cl_2$ was accomplished in 60-72% overall yield (Table 8). This method is highly labour intensive with a high potential for possible error and required 4-5 hours to complete. However, the quality of the product was excellent as judged by negligible losses after recrystallization.

b) Direct method: The synthesis of cis-platinum can also be accomplished from K_2PtCl_4 by its reaction with ammonia in buffered solution according to the following reaction:

$$K_2PtCl_4 + 2NH_4OH \longrightarrow \text{cis-}Pt(NH_3)_2Cl_2 + 2H_2O + 2KCl$$

Ammonium acetate has been used for this purpose, acting as both

buffer and ammonia source[613]. The acidity of the solution helps suppress the hydrolysis of the chloroplatinite and the slow release of ammonia from the salt decreases the possibility of tetrammine, triammine and Magnus salt ($[PtCl_4][Pt(NH_3)_4]$) formation. An alternative used here is the NH_4OH/NH_4Cl buffer reagent. The rate of reaction of $PtCl_4^{-2}$ with ammonia is proportional to the ammonia concentration, where as hydrolysis is relatively independent of hydroxide concentration and the best conditions have been found to be with NH_3:Pt ratios >4. The NH_4Cl limits the dissociation of NH_4OH and the chloride ions serve to hinder the formation of hydrolysis products, both decreasing the possibility of by-product formation. The above reaction takes place at slightly above neutral pH and at reduced temperatures[598]. Yields from this procedure were very poor (<10%) due to the noticeable formation of Magnus salt. Thus, although the method is extremely simple, the exacting conditions necessary for the successful synthesis appear to be lacking, possibly due to a pH and/or concentration effect.

Table 8. Chemical yields of cis-platinum from synthetic procedures.

Starting Material	Percent Yield in Trial Number 1	2	3	4	5
$H_2PtCl_6 \cdot 6H_2O$	40	45	58	55	48
K_2PtCl_4 - indirect method	60	68	72	65	65
- direct method	10	10	-	-	-

B. Purification and Analysis

The list of possible impurities from the synthesis of cis-platinum, by either the indirect or direct reactions are numerous but in practical terms, the most likely include elemental platinum, Magnus type complexes (mainly $[Pt(NH_3)_4][PtCl_4]$) and Pt(IV) chloroammines for the indirect method, while for the direct method trans-platinum, tetraammine and triammine salts could also be included.

Dissolution in 0.1 N HCl is preferred over water for recrystallization purposes since in this medium hydrolytic and aquation reactions will be suppressed. Many of the mentioned impurities are relatively insoluble in 0.1 N HCl so that this method of purification is somewhat effective. However, most of the undesirable compounds are electrolytes, whereas cis-platinum is a neutral complex such that more effective purification involves recrystallization from non-aqueous solvents. Cis-platinum is insoluble in all common organic solvents with the exception of DMF. Of the possible contaminants, only trans-platinum and the Pt(IV) diammine analogues are significantly soluble. The addition of two volumes of 0.1 N HCl combines the advantages of both methods. Elemental platinum and Magnus complexes are insoluble in DMF and can be removed by centrifugation or filtration before the addition of the 0.1 N HCl. Trans-platinum and the chloroammine electrolytes of Pt(II) and Pt(IV) are much more soluble in the DMF/0.1 N HCl mixture than in 0.1 N HCl alone and thus will remain in solution. Neutral Pt(IV) chlorodiammines behave similarly to cis-platinum but they will not crystallize from solution if present in low quantities[600].

Cis-platinum products were analyzed using the criteria of general appearance, specific chromatography (2 systems), physical (ultraviolet spectroscopy) and chemical behavior.

Pure cis-platinum appears as a bright yellow solid. The trans compound is a pale shade of yellow, Magnus complexes are usually green, tetrachloroplatinite is red-orange and elemental platinum appears grey or black. These substances can thus be identified in a crude mixture if present in sufficient quantities. However, most of the Pt(II) and Pt(IV) chloroammines are also yellow and could not be resolved by visual inspection. At certain stages during the indirect synthetic procedures elemental platinum has been observed and Magnus salts were readily observed during the direct synthesis from K_2PtCl_4. No anomolous compounds were observed after crystallization which is the final step before purification.

1. Chemical Test

The reaction of cis-platinum type compounds [diamminodihalogenoplatinum(II)] with thiosulphate has been used to determine the isomeric configuration of these complexes[602]. Trans-platinum reacts with thiosulphate at room temperature to form a disubstituted complex [trans-diamminedithiosulphateplatinum(II)] whereas cis-platinum forms a tetrasubstituted analogue due to the greater trans influence of the thiosulphate with respect to the chloride or ammonia ligands. The addition of traces of potash results in the formation of the corresponding potassium salts which are most characteristic. In practise, the cis-platinum product [$K_6Pt(S_2O_3)_4$] separates as an oily yellow liquid, and the trans- platinum product

$[K_2Pt(NH_3)_2(S_2O_3)_2]$ precipitates as a white crystalline solid. When this test was applied to any synthetically prepared cis-platinum after purification only the yellowish oil could be visibly detected. This qualitative test was applied only to aliquots of the first several syntheses since it required a relatively large sacrifice of product. However, it definitely confirmed that the vast majority of the product possessed the desired configuration.

2. Spectrophotometric Test

A typical ultraviolet spectrum recorded using freshly purified cis-platinum in 0.1 N HCl (1 mg/ml) is shown in Figure 6. The characteristic features of position and absorption of the major maxima and minima can be observed. These spectral features correspond to orbital electronic transitions in the d shell of the platinum atom which are involved in the binding process[614]. In terms of purity analysis, the most sensitive parameter is the ratio of the absorbance at the maximum (λ = 301 nm) to that at the minimum (λ = 246 nm). Since all Pt(II) and Pt(IV) chloroammine complexes absorb in the 246 nm region any slight increase in the absorbance due to these substances will have a marked effect on this ratio (and is often accompanied by shifts in the position of this minimum. The criterion for 'pure' cis-platinum using this index has been suggested as a ratio of 4.5 or greater[615]. All samples analyzed by U.V. spectroscopy which had been purified by recrystallization gave ratios of 4.3 ± 0.02 and were considered to represent satisfactory purity by this method.

3. Chromatographic Tests

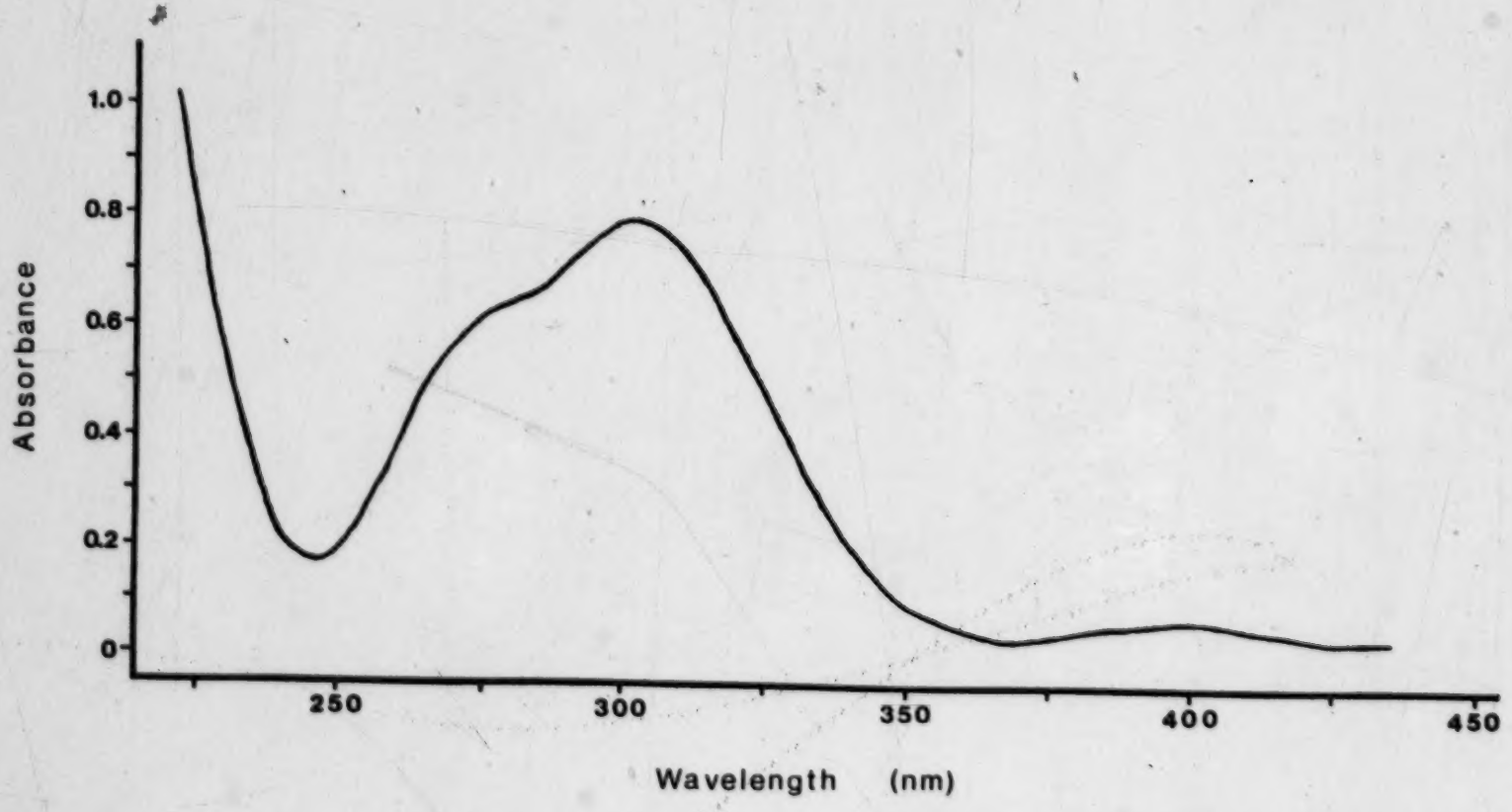


Figure 6. Ultraviolet absorption spectra of synthesized cis-platinum.

Results of the chromatographic analysis of cis-platinum and related compounds are shown in Table 9. Each of the platinum complexes showed a characteristic Rf value (Rf is the distance from the origin travelled by the test compound divided by the distance from the origin travelled by the solvent) in both systems which were readily reproducable. Using cellulose as the stationary phase the distinction between cis and trans isomers was most apparent, however, in both systems these compounds were easily resolved.

Fresh batches of cis-platinum consistently showed only one spot in both systems at its characteristic Rf, however, aged samples or those which had been inadvertently heated revealed the presence of compounds with an Rf corresponding to trans-platinum and another with an Rf between cis-platinum and K_2PtCl_4 which may be the trichloromonoammine derivative.

Table 9. Chromatographic analyses of cis-platinum and related compounds.

Compound	System I*		System II**	
	Rf (mean ± s.d.; n=8)	Colour+	Rf (mean ± s.d., n=6)	Colour+
cis-Pt	0.77 ± 0.02	yellow	0.80 ± 0.02	yellow
trans-Pt	0.60 ± 0.03	yellow	0.92 ± 0.02	yellow-brown
K_2PtCl_4	0.95 ± 0.03	gold	0.85 ± 0.02	brown-black
H_2PtCl_6	1.0	gold	-	-

* System I consisted of a cellulose stationary phase and a 0.2 M glycine solvent.
**System II consisted of a silica gel stationary phase and a acetone:water (9:1) solvent.
+ spot colour after I_2 visualization.

II. Detection of Platinum

There are a variety of techniques available for the detection of platinum. Gravimetric analysis involves weighing a known compound of platinum, such as the precipitated sulphides, the reduced metal or organic complexes. Titrimetric procedures involve reduction-oxidation or complexation reactions with specific agents. Platinum forms colored complexes with several reagents such as stannous chloride or para-nitrosodimethylaniline, forming the basis for the colorometric test. Electrochemical determinations involve polarography and coulometry[616]. All of these methods are basic analytical chemistry techniques and are not directly applicable to complex solutions or biological materials, where their lack of sensitivity and specificity make them inappropriate.

Three more modern techniques were evaluated for their applicability to the detection of platinum in a variety of simple and complex media. The most suitable method would hopefully be utilized for the detection of platinum deposition and its correlation with ^{67}Ga distribution in the system under study. All of the methods rely on the detection of the platinum portion of the cis-platinum molecule and are unable to assess its chemical environment. However, the active portion of the drug lies with the platinum atom, such that this distinction is insignificant in most cases.

A. Use of Labeled cis-Platinum

1. Synthesis

The availability of a suitable radioisotope production facility in the form of the Slowpoke II reactor as well as a viable synthetic

route allowed for the possibility of labeled compound preparation. Perhaps the best target for the production of a single useful platinum radioisotope is ^{194}Pt, which is activated to ^{195m}Pt during thermal neutron irradiation. However, this highly enriched material was not available for these studies and thus natural platinum materials were investigated. The larger proportion of platinum in K_2PtCl_4 compared to $H_2PtCl_6.6H_2O$ as well as its more favourable position in the synthetic sequence makes this compound the most logical starting material. Thermal neutron activation of K_2PtCl_4 produces numerous platinum radioisotopes (Table 10) as well as ^{38}Cl and ^{42}K. A four hour cooling period is sufficient to allow for the decay of ^{38}Cl (t 1/2 = 38 min) after which time the ^{42}K constitutes the major source of background activity. The ^{42}K is eliminated from the process during the reaction sequence but contributes to the shielding requirements. The 4 hour cooling period also results in the decay of ^{199}Pt (t 1/2 = 30 min) to its radioactive daughter, ^{199}Au, which is the most serious radiochemical impurity in the preparation. The ability of dibutyl sulphide (DBS) or methylisobutyl ketone (MIBK) to selectively extract gold from acidic solutions makes removal of this radiogold a simple procedure, leaving the radioplatinum as a solution of K_2PtCl_4. The limited success with the direct synthesis of cis-platinum from K_2PtCl_4 (Table 8) was such that only the indirect route was attempted for the labeled compound synthesis. The chemical yields of the labeled product are shown in Table 11, but no attempt was made to calculate radiochemical yields due to the low specific activity of the individual platinum radioisotopes in the complex.

Table 10. Production of platinum isotopes via thermal neutron irradiation of natural platinum[350].

Stable Nuclide A	% Natural Abundance	Cross-Section (Barns)	Nuclide Produced	$T_{1/2}$	Mode of Decay	Decay Product*
190	0.013	800	Pt-191	3.00 d	E.C.	Ir-191*
192	0.78	10	Pt-193	50 yr	E.C.	Ir-193*
		2	Pt-193m	4.33 d	IT	Pt-193
194	32.9	1.1	Pt-195	-	-	-
		0.09	Pt-195m	4.02 d	IT	Pt-195*
195	33.8	27	Pt-196	-	-	-
196	25.3	0.07	Pt-197	18.3 hr	β^-	Au-197*
		0.05	Pt-197m	94.4 min	IT	Pt-197
198	7.2	3.7	Pt-199	30.8 min	β^-	Au-199
		0.03	Pt-199m	14.1 sec	IT	Pt-199

* refers to stable isotope

Table 11. Chemical yields of labeled cis-platinum from preparation procedures.

Method	Percent Yield in Trial Number 1	2	3
Synthesis from $[K_2PtCl_4]$*	53	65	60
Direct Activation	>95+	>95+	>95+

* $[K_2PtCl_4]$ neutron activated complex

+ based on usuable cis-platinum at the end of sample processing

2. Direct Activation

With the possibility of avoiding the time consuming radiochemical synthesis and increasing the specific activity of the product, a second approach to the preparation of the labeled compound involving the direct bombardment of cis-platinum was investigated. Irradiation of purified cis-platinum for 4 hour at 1 x 10^{12} n.cm^{-2} sec^{-1} resulted in the formation of a black material from the bright yellow powder. Since the compound is totally inorganic this effect was not charring and indicated another form of degradation was occurring *in situ*. Capsule site temperatures will reach 40°C during bombardment, however, thermal decomposition of cis-platinum does not occur until 207°C. This effect was not observed after irradiations in the outer capsule site for 2 hours and it is possible that the increased gamma and neutron flux of the inner site was responsible for a Szilard-Chalmers reaction forming elemental black platinum. Several other platinum compounds have also been shown to undergo this reaction under similar conditions[617].

Direct activation of the complex in this manner necessitated removal of ^{199}Au and a post-irradiation purification procedure by specific recrystallization. The chemical yield of the labeled product is shown in Table 11 as greater than 95% since recrystallization losses are generally small.

3. Purification and Analysis

Since the specific activity of the labeled cis-platinum prepared by either synthetic reactions from K_2PtCl_4 or by direct activation was very low, all the chemical and physical analyses which were applicable

to the unlabeled compound would be equally suitable here. The chemical test was not performed on the labeled compound since it required considerable quantities of material. The spectrophotometric test was performed on a single batch of labeled cis-platinum prepared by direct activation and the resultant absorbance ratio was calculated as 4.3, indicating adequate purity. The majority of testing of the labeled compound was conducted using the more rapid chromatographic assays developed for the stock material, with additional analysis of radiochemical parameters.

Radiochemical analysis of labeled cis-platinum is shown in Figure 7. These chromatographic scans reveal the presence of a single radioactive peak in most cases, at an Rf corresponding to the pure unlabeled product in both systems. The estimated radiochemical purity from quantitative data is greater than 90%, with segmental counting of paper chromatograms revealing essentially the same information as shown in Table 12. No anomalous radioactive peaks were observed in these chromatographic systems, confirming the radiochemical purity of the labeled cis-platinum.

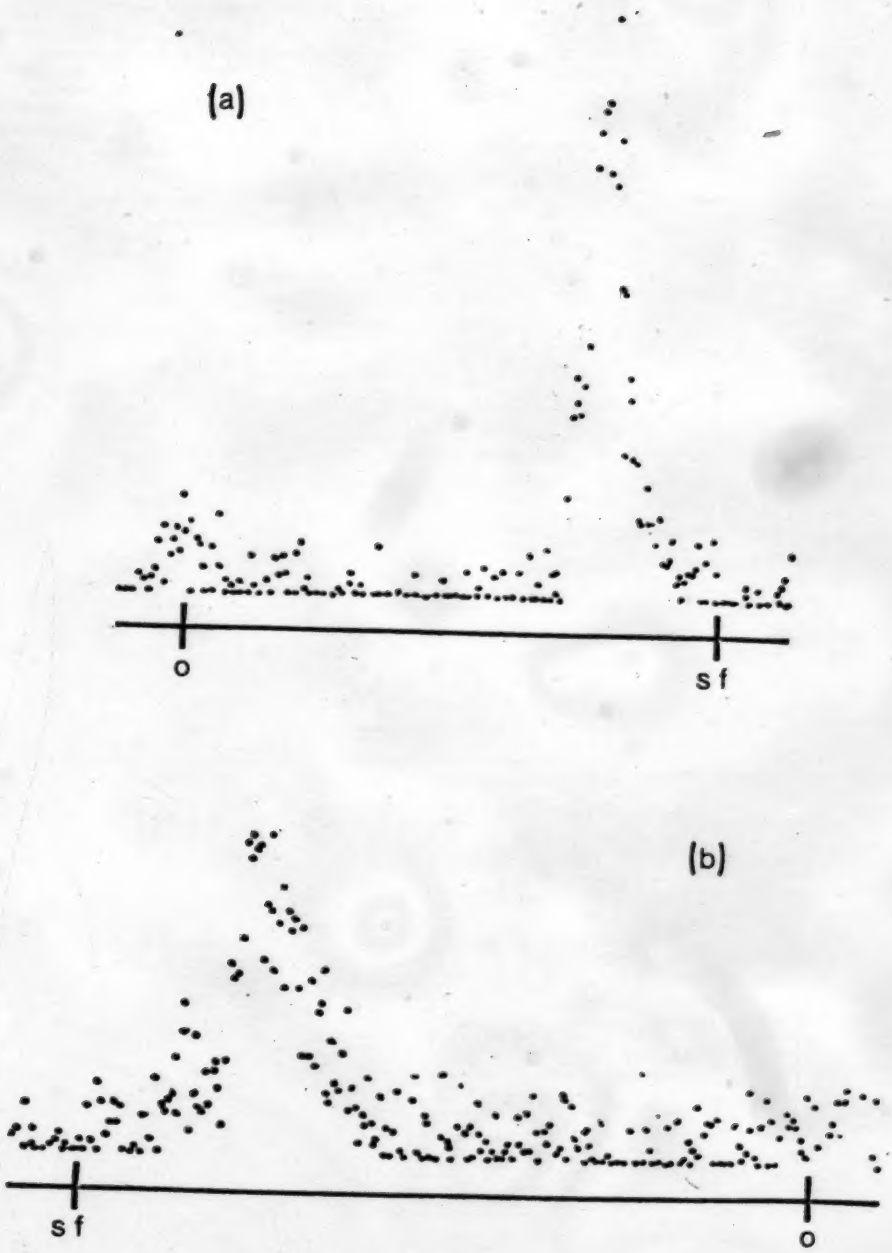


Figure 7. Radiochromatographic analysis of labeled cis-platinum using (a) a silica gel stationary phase and an acetone:water (9:1) solvent and (b) a cellulose stationary phase and a 0.2 M glycine solvent. (0 = origin ; sf = solvent front)

Table 12. Radiochromatographic analysis of labeled cis-platinum.

Analysis method	Cis-Pt Rf (mean ± s.d.; n=4)	% activity in cis-platinum peak (mean ± s.d.; n=4)	Chromatographic System*
Whole Strip Scan	0.76 ± 0.02	93 ± 4%	I
Strip Segments Histogram	0.73 ± 0.09	>94%	I
Whole Strip Scan	0.80 ± 0.02	91 ± 2%	II

*System I consisted of a cellulose stationary phase and a 0.2 M glycine solvent.
System II consisted of a silica gel stationary phase and an acetone:water (9:1) solvent.

4. Counting of Labeled cis-Platinum

The characteristics of platinum radioisotopes produced via thermal neutron irradiation of ntural platinum are shown in Table 13. The basic problem concerns which of the eight platinum radioisotopes produced in this manner is most suitable in terms of external detection properties over the duration of a study. Only ^{191}Pt, ^{193m}Pt, ^{195m}Pt and ^{197}Pt would be useful for experiments longer than 24 hours, and of these ^{191}Pt and ^{197}Pt are produced in the greatest yields.

Table 13. Emission characteristics of platinum radioisotopes produced from neutron irradiation[350].

Radioisotope	Decay Mode	$T_{1/2}$	Emissions in KeV (% Absolute Abundance)
Pt-191	E.C.	3.00 d	γ:539(13.7);409(8);360(6) Ir LX(36); Ir KX(138) e^-:20;53;69;80 (others between 0 and 600)
Pt-193	E.C.	~50 yr	none
Pt-193m	IT	4.33 d	γ:136(0.11) e^-:10;57;124;133
Pt-195m	IT	4.02 d	γ:99(11.1);130(2.9) Pt KX (87.2) e^-:18;28;51;85;116;126
Pt-197	β^-	18.3 hr	γ:77(17);191(3.5) β^-:719(11);642(80);451(10) e^-:63;74;110
Pt-197m	IT	94.4 min	γ:347(12) e^-:40;50;268;332
Pt-199	β^-	30.8 min	γ:542(15) β^-:1690(63);1380(4); 1140(14);900(18)
Pt-199m	IT	14.1 sec	γ:392(84.7)

a) Gamma counting: For NaI(Tl) gamma counting purposes ^{191}Pt produces a 539 KeV photon, however, it is of low abundance (13.7%) and would be subject to interference from spurious 511 KeV photons. ^{197}Pt emits a low energy gamma (77 KeV) in fair abundance (17%) and a second at 191 KeV, but the first cannot be resolved from numerous X-rays and the second has low abundance (3.5%). For ^{193m}Pt, X-rays are the only significant gamma emissions, while ^{195m}Pt photons are also subject to X-ray interference at 99 KeV or of low abundance (130 KeV; 2.9%).

The major gamma emissions of an irradiated platinum sample are shown in Figure 8a, as recorded by a 3" x 3" NaI(Tl) well detector. The largest peak corresponds to Pt, Au and Ir X-rays generated by the decay of ^{191}Pt, ^{193m}Pt, ^{195m}Pt, ^{197}Pt and ^{199}Au with contributions from the 77 KeV and 99 KeV gamma of ^{197}Pt and ^{195m}Pt, respectively. The second largest peak at 159 KeV is due to ^{199}Au and the third peak at about 200 KeV is due ^{199}Au (208 KeV) and ^{191}Pt (191 KeV). Extraction of the radiogold results in the spectrum shown in Figure 8b. Residual radiogold in the aqueous phase ($<5\%$) is likely due to the slightly reduced extraction efficiencies using carrier free conditions. Thus, the X-ray peak is responsible for the vast majority of useable photons from the decay of platinum radioisotopes produced from thermal neutron irradiation. The relative isotope composition of this isotope peak (calculated from literature data) indicates that ^{191}Pt contributes almost 80% of the observed X-rays with the rest due to ^{193m}Pt and ^{197}Pt. Verification of this composition was obtained by stripping the observed decay curve (Figure 9b), indicating the majority of activity is due to isotopes with half-lives corresponding to ^{191}Pt (3.0 d), ^{195m}Pt (4.0 d) and ^{197}Pt (18.3 hrs).

Use of the X-ray peak for counting purposes necessitates the removal of ^{199}Au from the preparation since it generates Hg X-rays with fair abundance (17%). Since the useable X-ray peak is of multiple isotope composition, the decay correction is not easily calculated empirically. Thus, counts generated in this peak from test samples were correlated to standards counted before and after each sample and corrected to a common value.

b) Liquid scintillation counting: A second approach to detection

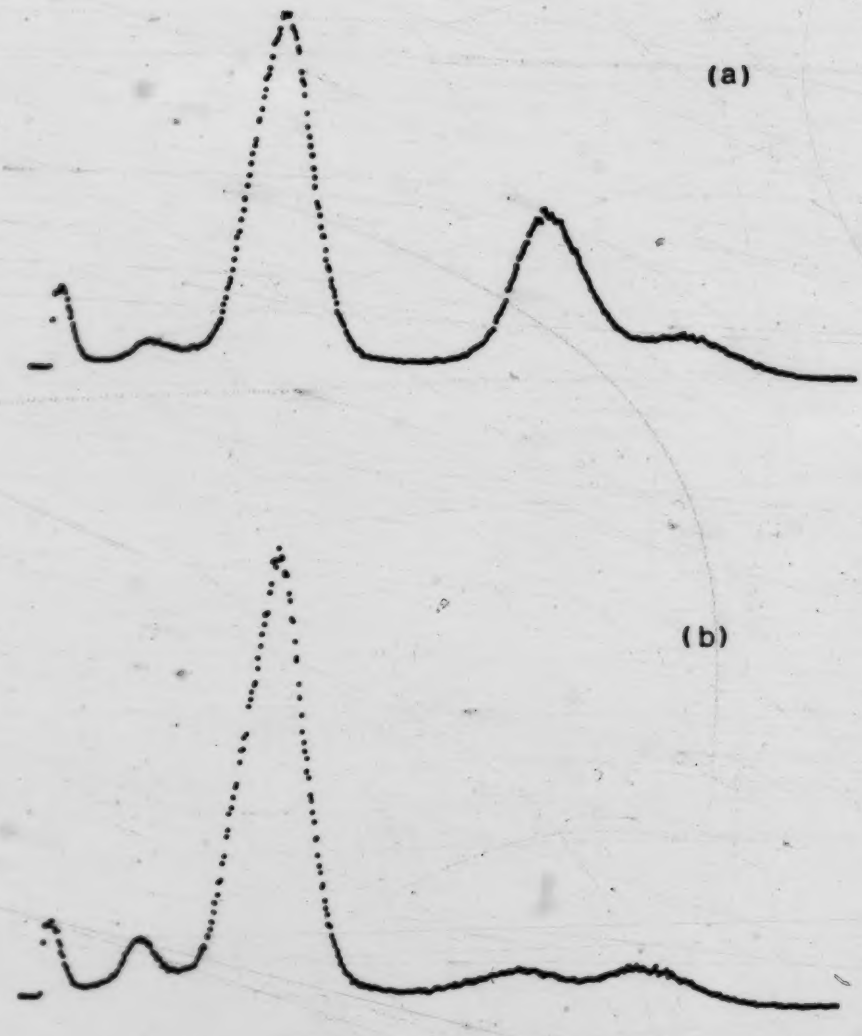


Figure 8. Relative NaI(Tl) detector gamma spectrum of radiolabeled cis-platinum (a) before and (b) after radiogold (Au-199) separation.

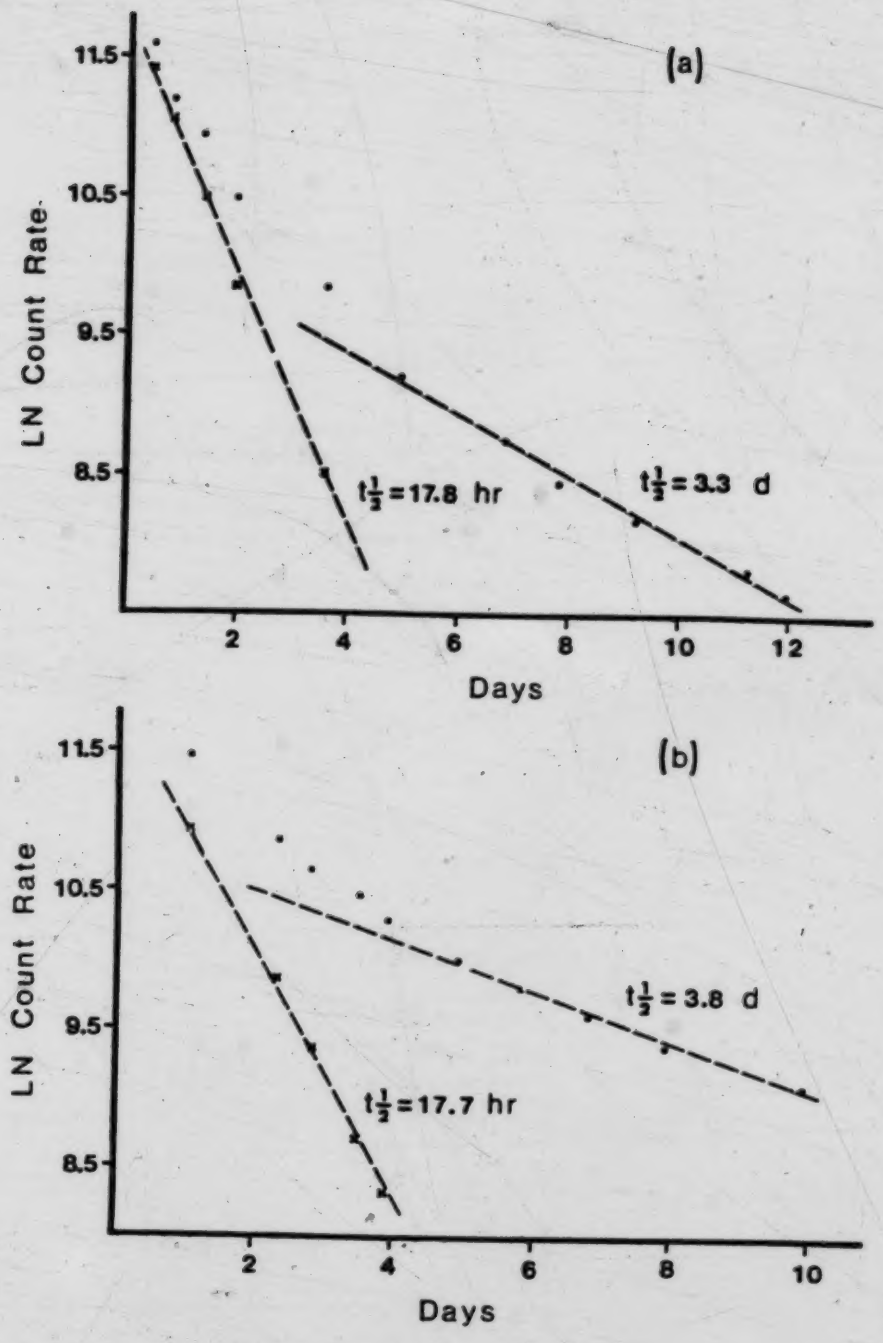


Figure 9. Resolved decay curves of radiolabeled cis-platinum measured by (a) liquid scintillation counting and (b) gamma counting of the X-ray peak.

of the labeled compound via liquid scintillation (LS) was also investigated. The platinum radioisotopes produced in the reactor which have useful half-lives all decay with the particle emissions, either as β^- particles (^{197}Pt) and/or conversion electrons (^{191}Pt, ^{193m}Pt, ^{195m}Pt, ^{197}Pt) of various energies (Table 13). These particles generate light photons (scintillations) within the fluor solution in which the sample is dissolved and are electronically detected by the LS instrument. The LS spectra of the irradiated compound is shown in Figure 10, with standard isotope samples recorded under the same conditions for comparison. At high particle energies the log scale produces considerable insensitivity and makes accurate energy determinations difficult. However, it appears that the cut-off energies of the spectra correspond to the expected β^- max energies. Decay curves of the observed LS data is shown in Figure 9a. Similar to the X-ray peak decay curve, these data confirm the presence of ^{197}Pt and the three longer lived radioisotopes ^{191}Pt, ^{193m}Pt, ^{195m}Pt as the source of the activity.

The LS counting proved to be a more efficient method for the detection of the labeled compound by a factor of 3-4, compared to X-ray peak counting. This is due to a combination of the greater number of countable emissions and the increased detection efficiency of the LS system for these emissions. ^{199}Au is also counted with high efficiency and cannot be distinguished from platinum radioisotopes via spectral analysis as in the case of the NaI(Tl) detector where resolution of the X-ray and 159 KeV peaks is possible. Thus, residual ^{199}Au in the preparation would lead to improper tracer detection and erroneous sample counts. For this reason, gamma counting was deemed

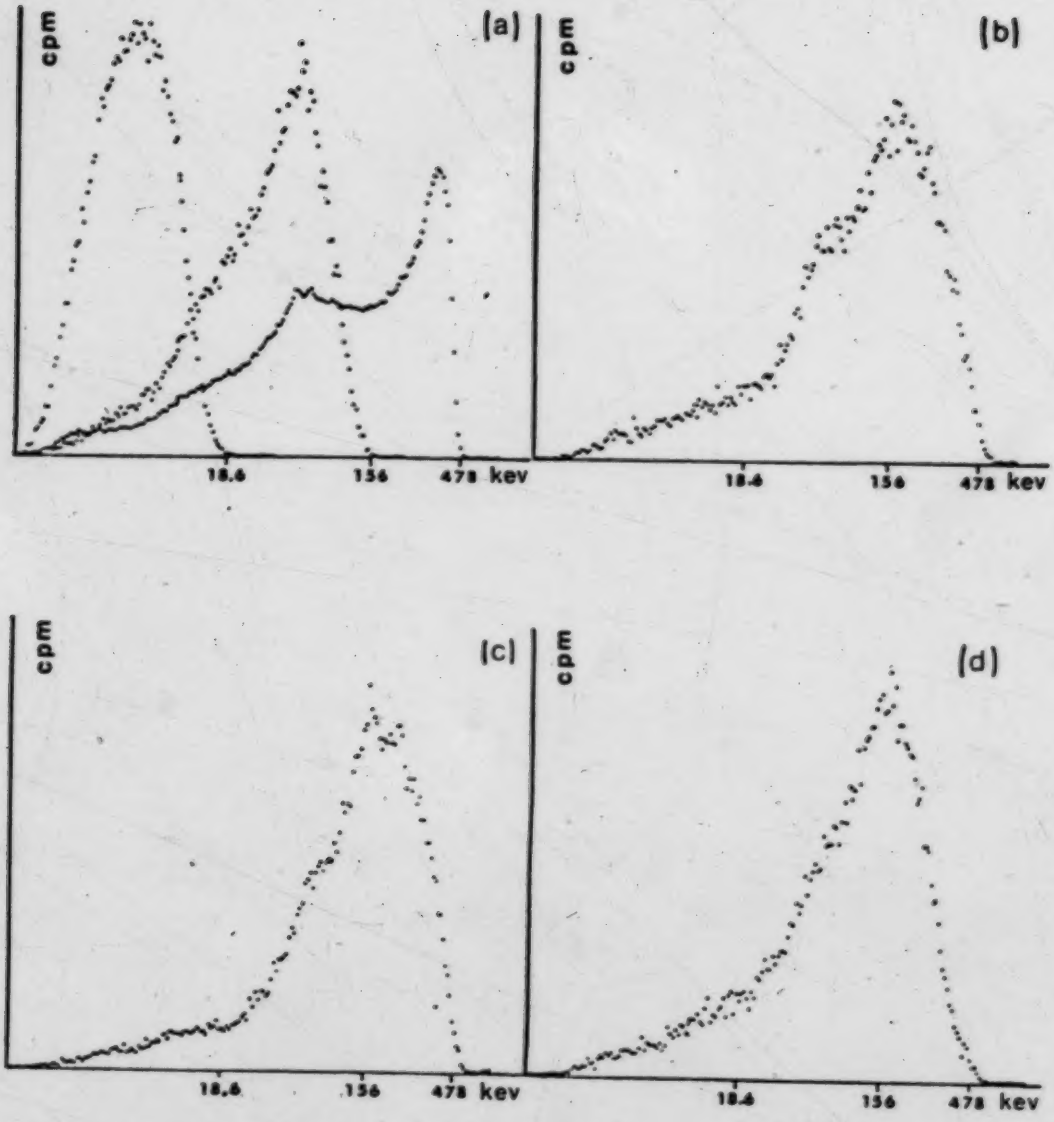


Figure 10. Liquid scintillation spectra of (a) a standard mixture containing H-3, C-14, and Cs-137 ; radiolabeled cis-platinum (b) before and (c) after radiogold (Au-199) separation ; (d) radiogold Au-199.

to be the method of choice for counting labeled cis-platinum samples.

B. Atomic Absorption Spectrometry

Atomic absorption spectrophotometry is based on the specific energy absorption of free atoms at highly characteristic wavelengths and has been used for the quantitative determination of almost all metals in the periodic table. Analytical methods for platinum by atomic absorption spectrophotometry were developed largely for ore and alloy analysis but have more recently been applied to biological samples.

Operating conditions for platinum determinations using the Perkin-Elmer 305B instrument were chosen using the manufacturer's recommendations. Simple buffered or salt solutions of cis-platinum were readily analyzed by standard procedures.

Samples containing a large proportion of organic material have been shown to generate anomalous readings due to the physical nature of the process. During the char phase, profuse smoking of this material occurs within the furnace which results in excessive non-atomic absorption during the proceeding atomization phase, and deuterium arc background correction becomes inadequate. Attempts to reduce this effect have taken the form of temperature adjustments using an available instrument accessory. In this way, more gradual temperature increases are possible, such that a sufficient time interval between the appearance of the smoke signal and the platinum signal eliminates any additive peaks. This method has been used for platinum analysis of human serum and urine[618,619].

Attempts to analyze other biological material have all focused on

sample digestion techniques proposed to eliminate the considerable matrix effects due to organic residue. Wet ashing of tissues with 70% HNO_3 or $HNO_3/HClO_4$ combinations have been employed and a more recent technique utilizes total sample combustion. Exact conditions must be maintained in these procedures in order to ensure complete recovery of platinum and reproduceability of the assay. The detection limits by these methods have been estimated as about 100 ng/g (wet weight) of tissue[620].

The analysis of platinum by atomic absorption spectrophotometry was utilized for simple aqueous solutions due to the ease and sensitivity of the procedure, however, we believed the development of a neutron activation analysis technique would be more suitable for biological tissue samples.

C. Neutron Activation Analysis

Although flameless atomic absorption spectrophotometry was suitable for the analysis of platinum in simple solutions and the labeled compound would be useful for short term in vitro determinations, a method of detection which could quantitate relatively low levels of platinum in a complex biological matrix was sought.

The assay of platinum by neutron activation analysis is based on the production and selective isolation of radiogold. Thermal neutron activation of platinum results in the formation of eight radionuclides of platinum and a radiogold daughter via $^{198}Pt(n,\gamma)^{199}Pt \rightarrow {}^{199}Au$. The advantages of using this second order reaction product as the marker isotope for platinum analysis are numerous. ^{199}Au is produced in good

yields during irradiation and has a relatively long half-life which permits the conduct of post-irradiation radiochemical procedures after the decay of shorter-lived products and allows for extended counting intervals. It decays with adequate photon abundance of sufficient energy to be reliably counted above the numerous gamma emissions present at lower energies. The most important characteristic of this isotope for this application is that its chemical nature enables selective extraction from a host of interfering activated products. Also, a non-interfering long-lived radiogold isotope is commercially available (^{195}Au) to act as a tracer for the ^{199}Au extraction yield calculation. The important physical characteristics of these isotopes are shown in Table 14.

Table 14. Characteristics of radiogold isotopes used in the determination of platinum by neutron activation analysis[350].

Measured Isotope	$T_{1/2}$	Major Gamma Emissions	
^{195}Au	182.9 d	98.9 KeV	11%
		67 KeV	89%
^{199}Au	3.15 d	158.4 KeV	36.8%
		208.2 KeV	7.7%

Although a variety of organic solvents are capable of efficiently extracting chloroaurates from aqueous solutions, the most specific are the dialkyl sulphides. Virtually no other metallic elements are co-extracted with gold under the appropriate conditions[621]. Extraction efficiencies greater than 98% have been obtained from simple solutions using these agents and were found to be

relatively independent of acidity (0.1-6.0 M)[622] and organic to aqueous solvent ratio (<20:1)[623]. Recovery from digested biological material by this method is generally about 90% and the exact yield for each sample is calculated from ^{195}Au extraction measurements which are used for data correction.

There are numerous acidic mixtures capable of solubilizing organic material by oxidative degradation. Combinations of HNO_3, $HClO_4$ or H_2O_2 with H_2SO_4 have been used in this respect for the digestion of biological material. In each case, solubilization of a standard 100 mg sample of freeze dried plasma could be accomplished in a reasonable period of time (~2 hrs), however, the heating requirements with respect to removal of residual oxidant and loss of radiogold during digestion were variable. Total removal of oxidant by the end of digestion was found to be necessary since the disulphide extractant is highly susceptible to oxidative degradation. Both HNO_3 and $HClO_4$ form high boiling point azeotropes with residual water and thus high temperatures are required for their complete removal (these azeotropes boil at 120°C and 203°C, respectively)[610]. This heat factor would not be prohibitive were it not for the observed loss of radiogold from digesting solutions of this compositions at these temperatures. Significant losses (Table 15) were observed at temperatures >110°C and thus neither HNO_3 or $HClO_4$ (with H_2SO_4) were deemed suitable in this application. Although elemental gold is volatile at these temperatures, the losses observed after vapour collection and condensation indicate a soluble species and is likely due to simple loss of all solution components via the boiling process. The use of a peroxide-H_2SO_4 system provides for a low temperature

digestion process with total removal of residual oxidant by peroxide decomposition. The H_2SO_4 provides the acidic medium necessary for the oxidation of gold to the Au(III) state by H_2O_2 (gold is reduced to the metal by peroxide under alkaline conditions)[624] and the subsequent addition of HCl serves to complex the oxidized gold forming the extractable product.

Table 15. Effect of temperature on radiogold loss during sample digestion in the determination of platinum by neutron activation analysis.

	Percent ^{195}Au Lost (n=3)					
Temp.	Duration of Digestion (hrs)					
(°C)	1	1 1/2	2	3	6	18
83	1.1±0.4	-	-	0.8±0.3	1.2±0.8	1.6±0.6
110	-	-	-	-	1.6±0.6	2.0±0.6
135	13.3±1.2	16.4±3.4	17.6±0.2	20.6±1.2	-	-
160	16.6±1.7	-	21.2±2.6	18.9±1.8	-	-

The only detectable element co-extracted with radiogold by dibutyl sulphide under these conditions is radiobromine (^{82}Br). The high affinity of bromine for such solvents is confirmed by its presence in many reagent grade synthetic dialkyl sulphides[625]. The results of adding carrier NaBr prior to sample digestion is shown in Table 16. The purpose of the NaBr was to act as a carrier for the conversion of radiobromide ($^{82}Br^-$) to radiobromine ($^{82}Br_2$) under these highly oxidizing conditions and the evolution of the gaseous product

from the hot solution. The addition of 200 mg and 500 mg NaBr results in a 95% and 99% reduction in radiobromine extraction respectively, and both appear to increase the variability of radiogold extraction possibly due to the variable formation and extraction of $AuBr_4^-$. At the 500 mg level, NaBr also reduces radiogold extraction and thus 200 mg NaBr was chosen to minimize radiobromine breakthough and maintain the high radiogold extraction efficiency.

Table 16. Effect of carrier NaBr on radiobromine and radiogold extraction in the determination of platinum by neutron activation analysis.

	Extraction Efficiency (%) (n=3)	
	^{195}Au	^{82}Br
Control (no NaBr)	87.4 ± 1.1	7.6 ± 1.0
200 mg NaBr	85.5 ± 10.7	0.4 ± 0.1
500 mg NaBr	73.9 ± 8.6	0.1 ± 0.1

The activation of calcium, which is endogenous to many biological samples, leads to the second order formation of ^{47}Sc. This daughter decays with the emission of a 159 KeV gamma which would be counted under the 158 KeV photopeak of ^{199}Au. However, trials with spiked calcium samples showed that neither ^{47}Ca nor ^{47}Sc appeared in the sample extract (verifying the specificity of the extraction). The large burn-up cross-section of ^{198}Au allows for the production of ^{199}Au in the absence of any platinum in the sample. However, using

irradiation conditions of 1.1×10^{13} $n.cm^{-2}.sec^{-1}$, the $^{199}Au/^{198}Au$ ratio has been found to be less than 2%[626] and no ^{199}Au activity was observed by this reaction even in high activity ^{198}Au samples and thus no correction was necessary in this case. The presence of any of the numerous other radionuclides with gamma emissions of comparable energy to the measured peaks of ^{195}Au or ^{199}Au could not be demonstrated to be present by analysis of the sample extract spectra and half-life considerations.

This post-irradiation solvent extraction technique effectively separates the radiogold from the vast amount of background activity, which is almost exclusively ^{24}Na, induced in the biological sample. The total process can be carried out in a well shielded fume hood to minimize personnel radiation exposure. Analyte loss due to handling and transfer have been eliminated by utilizing filter paper sample containers which are irradiated and digested together with the sample, and by conducting the whole process in a single test tube. The method readily lends itself to rapid batch analysis because it is convenient to digest numerous samples simultaneously. A dozen samples were easily processed in less than 2 hours. The calculated detection parameters indicate that approximately 60 ng of platinum can be reliably quantitated in a biological matrix using the above technique[627].

D. Summary

Three methods were evaluated for the detection of platinum from cis-platinum. The labeled compound was best prepared by direct bombardment of cis-platinum but the material suffered from low

specific activity and a rather inefficient counting situation. This method of detection of cis-platinum would have been most useful for dual label binding studies with ^{67}Ga but low activity and crossover would remain obstacles. The determination of platinum by neutron activation analysis was developed into a relatively simple and sensitive technique. It required sample preparation, reactor access, a decay period, an extraction process and sample counting time before results could be obtained. It would be the method of choice for low level detection of platinum in complex biological samples such as tissues. Atomic absorption spectrometry was best suited to the rapid analysis of simple solutions of moderate platinum concentration. Thus, it was the method of choice for the determination of platinum binding to transferrin in light of the presence of such conditions.

III. The Effects of cis-Platinum on the Biological Behavior of ^{67}Ga-citrate

A. Initial Observations on the Effects of cis-Platinum on ^{67}Ga-citrate Distribution in the Rat

The effects of cis-platinum treatment using a typical human dosage schedule on the tissue distribution of a subsequently administered injection of ^{67}Ga-citrate was evaluated initially using the rat as the *in vivo* model. The observed tissue-to-blood concentration ratio of ^{67}Ga for several major organs at various times after tracer injection are shown in Figure 11a-e. In the control animals, the ratio increases for a period of about 3 days and appears to reach a steady-state after this time. Data from animals treated with cis-platinum produced ratios which continued to increase even after 3 days, until at 7 days post ^{67}Ga-citrate injection the concentration ratios were significantly greater than control values. This effect was observed in most tissues examined, indicating a general pattern for this somewhat slow developing consequence of cis-platinum treatment. The second difference between control and treated animals occurred at the shortest time period studied. The tissue concentration ratio at 3 hours was higher than control values after cis-platinum treatment in all tissues where the difference was significant. Thus, the observed effect of cis-platinum treatment could be summarized in terms of an early and late consequence, both of which tend to increase the tissue-to-blood concentration ratios of ^{67}Ga in the rat. This is readily observed by the summary of data given in Table 17.

In this study, ^{67}Ga-citrate was administered concurrent with the

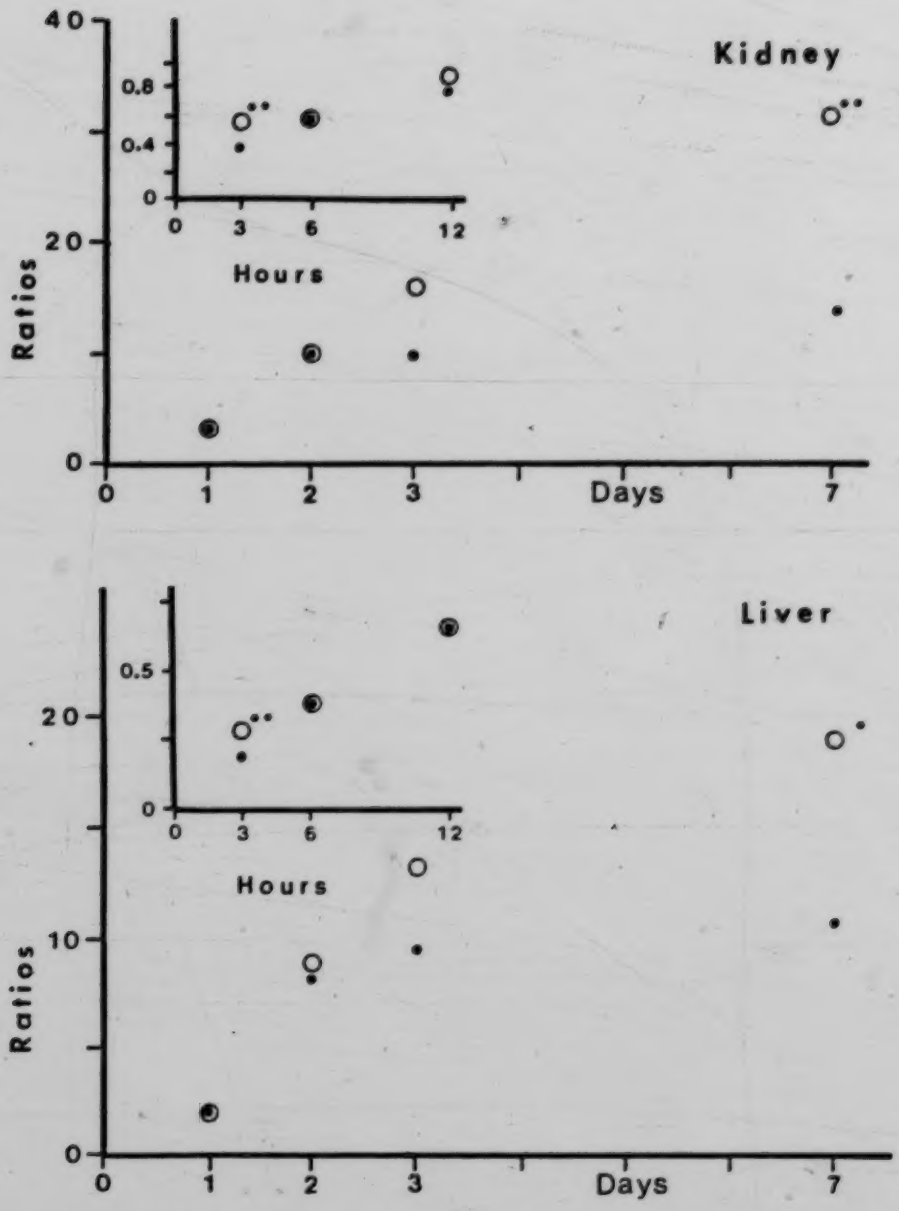


Figure 11a. Selected tissue to blood ratios of Ga-67 in control (•) and cis-platinum treated (O) Sprague-Dawley rats at various times after Ga-67 citrate administration. (only the mean values of 2-4 animals are shown; statistical significance is indicated by $^{*}p < 0.05$; $^{**}p < 0.01$)

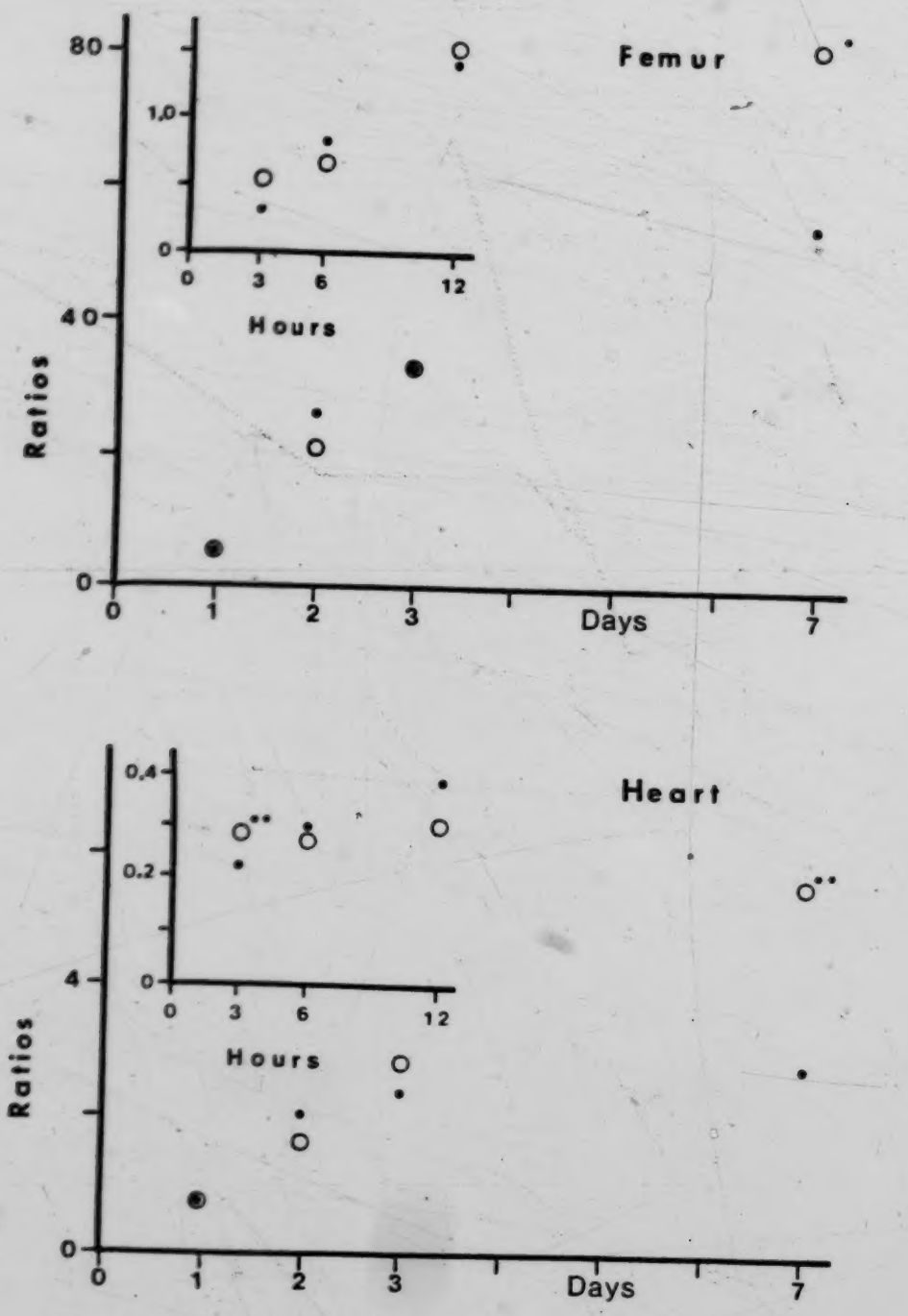


Figure 11b. Selected tissue to blood ratios of Ga-67 in control (•) and cis-platinum treated (O) Sprague-Dawley rats at various times after Ga-67 citrate administration. (only the mean values of 2-4 animals are shown; statistical significance is indicated by $^{*}p < 0.05$; $^{**}p < 0.01$)

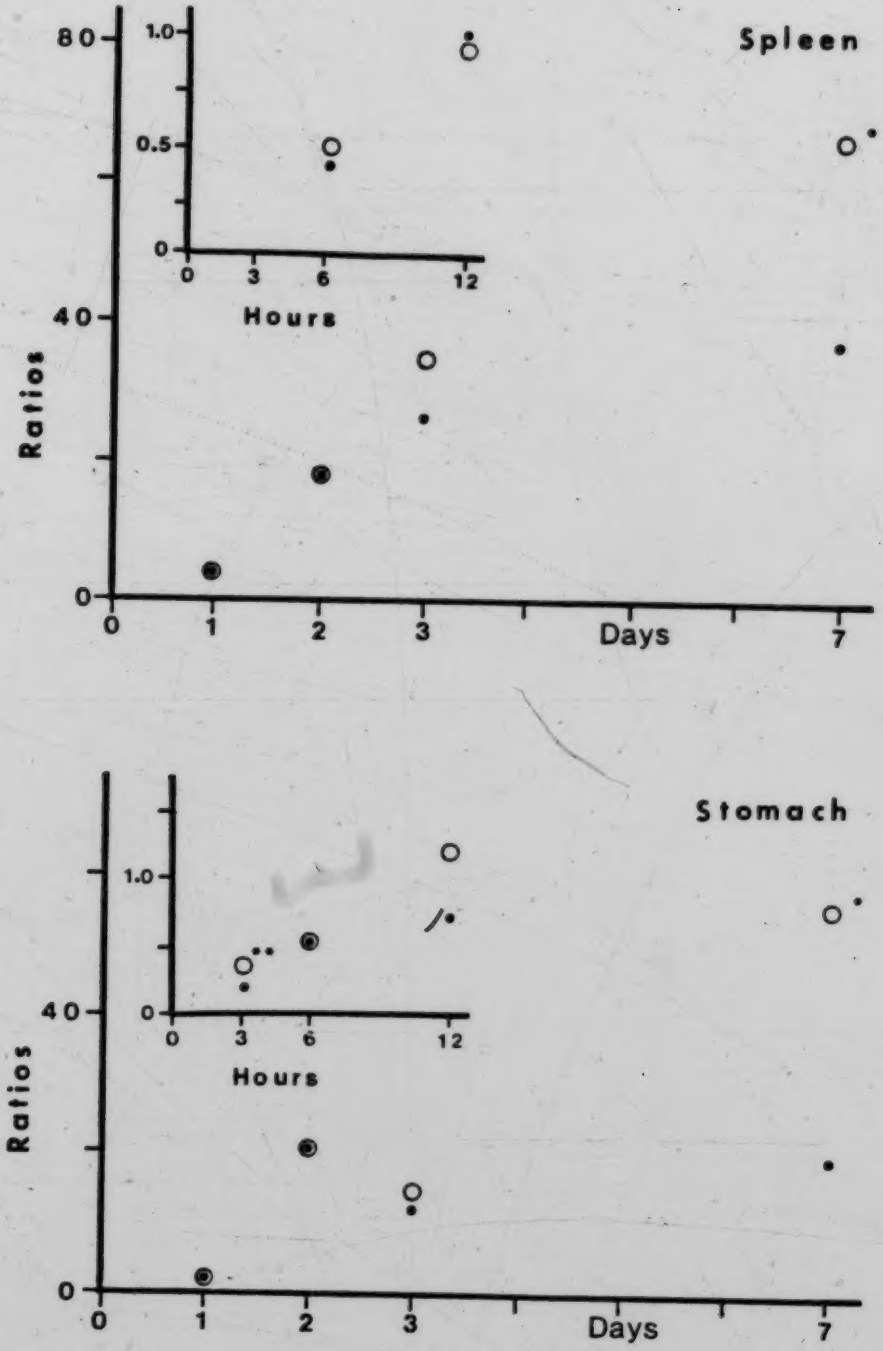


Figure 11c. Selected tissue to blood ratios of Ga-67 in control (•) and cis-platinum treated (O) Sprague-Dawley rats at various times after Ga-67 citrate administration. (only the mean values of 2-4 animals are shown; statistical significance is indicated by $^{*}p < 0.05$; $^{**}p < 0.01$)

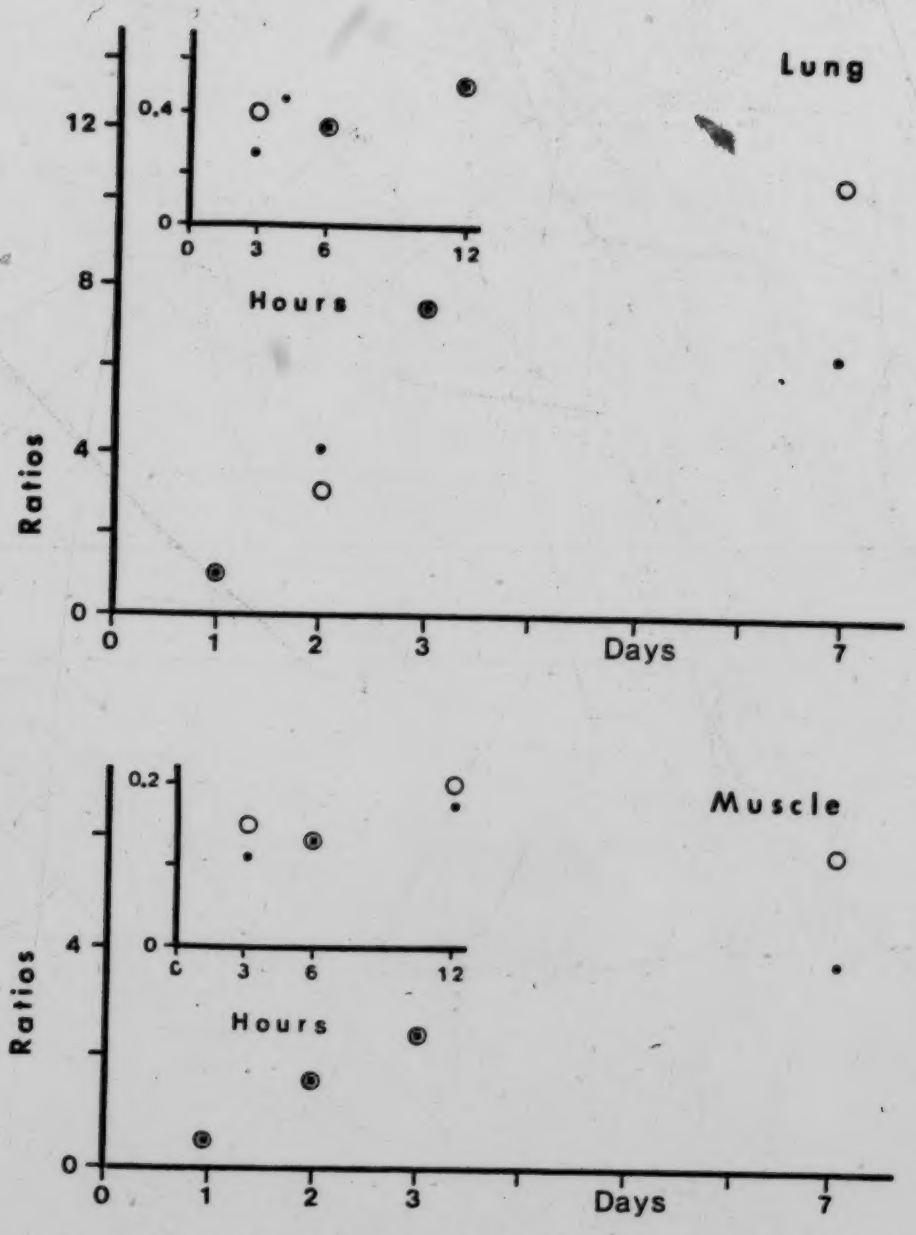


Figure IId. Selected tissue to blood ratios of Ga-67 in control (•) and cis-platinum treated (O) Sprague-Dawley rats at various times after Ga-67 citrate administration. (only the mean values of 2-4 animals are shown; statistical significance is indicated by $^{*}p < 0.05$; $^{**}p < 0.01$)

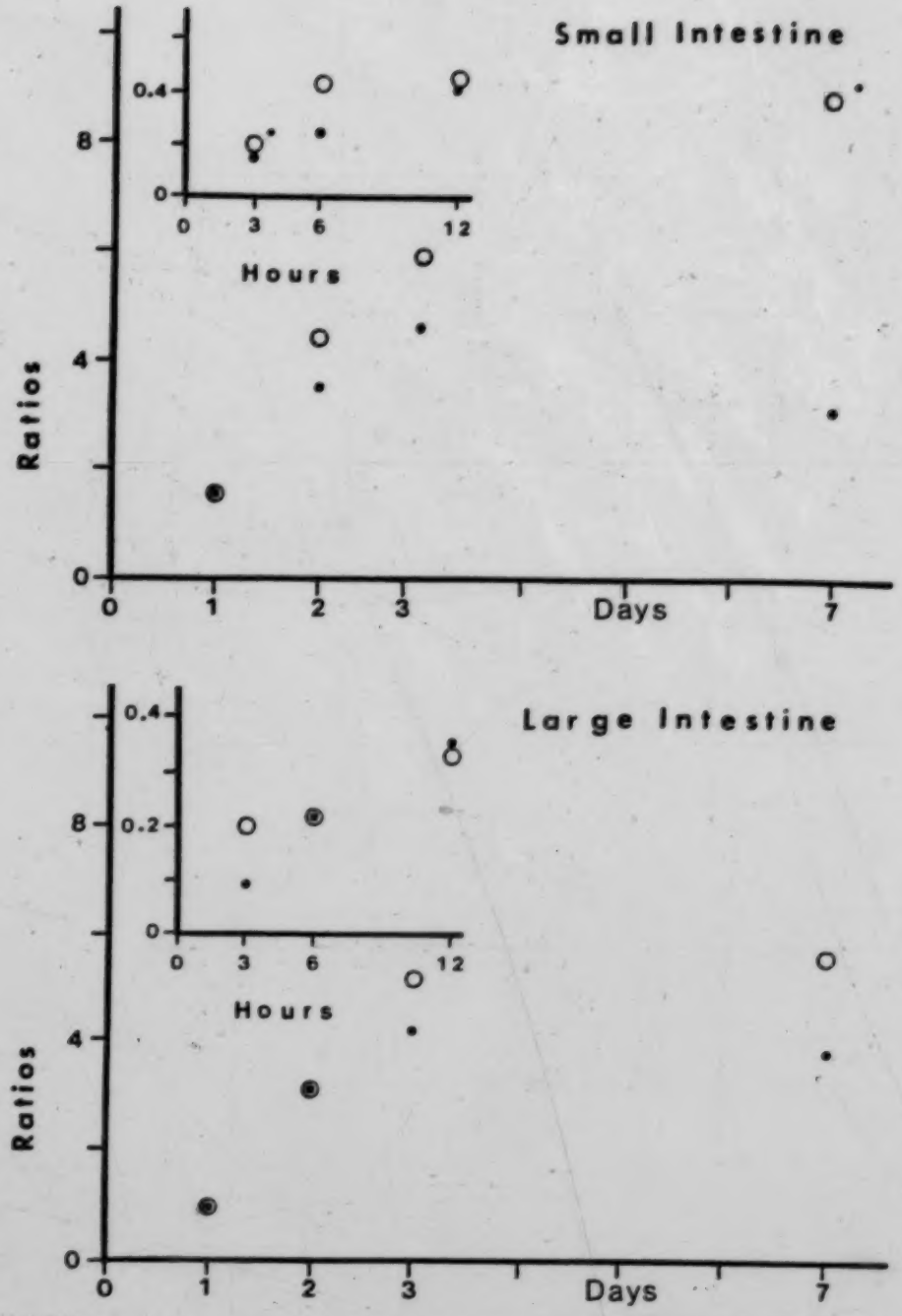


Figure 11e. Selected tissue to blood ratios of Ga-67 in control (•) and cis-platinum treated (O) Sprague-Dawley rats at various times after Ga-67 citrate administration. (only the mean values of 2-4 animals are shown; statistical significance is indicated by $^{*}p < 0.05$; $^{**}p < 0.01$)

Table 17. Summary of the effect of cis-platinum treatment on the tissue-to-blood ratios* of ^{67}Ga in Sprague Dawley rats at various times after ^{67}Ga-citrate administration.

	Level of significance relative to control group in unpaired t test (n=2-4)						
Tissue	3 hr	6 hr	12 hr	24 hr	48 hr	72 hr	168 hr
Bone	-	-	-	-	-	-	$p < 0.05$ (+)
Heart	$p < 0.005$ (+)	-	-	-	-	-	$p < 0.005$ (+)
Large Intestine	-	-	-	-	-	-	-
Small Intestine	$p < 0.0025$ (+)	-	-	-	-	-	$p < 0.025$ (+)
Kidney	$p < 0.01$ (+)	-	-	-	-	-	$p < 0.05$ (+)
Liver	$p < 0.005$ (+)	-	-	-	-	-	$p < 0.05$ (+)
Lung	$p < 0.025$ (+)	-	-	-	-	-	-
Muscle	-	-	-	-	-	-	-
Spleen	-	-	-	-	-	-	$p < 0.05$ (+)
Stomach	$p < 0.01$ (+)	-	-	-	-	-	$p < 0.05$ (+)

* cpm per gram of tissue divided by cpm per ml of blood
Note: (+) indicates cis-platinum increased ratio

last daily dose of cis-platinum on the fifth day of the treatment schedule. The early changes may thus represent an immediate effect of cis-platinum on the initial tissue uptake and excretion of the tracer mediated by a protein binding interaction. If cis-platinum was inhibiting the plasma protein binding of ^{67}Ga more unbound tracer would be available for clearance from the blood either by excretion processes or tissue uptake, both of which would account for the observed data. This was postulated to be due to a similar consequence to that caused by stable gallium, scandium, or iron administration, all of which cause rapid blood clearance of the tracer via renal excretion[391,392,416].

The increased concentration ratios observed one week after ^{67}Ga administration likely represent the long term effects of cis-platinum treatment on physiological function in the rat. Apparent kidney damage was observed in all rats sacrificed at this time period in concert with the known renal toxicity of the compound. Thus, impaired ^{67}Ga elimination by this route should lead to reduced blood clearance and decreased ratios. However, it may be that in the absence of sufficient competition from excretion processes, tissue uptake is also enhanced and to a greater extent relative to blood clearance depression.

This initial study in rats clearly demonstrated an effect of cis-platinum upon ^{67}Ga localization. However, it did not differentiate the effects of tissue concentration changes from those of the blood since the results could only be expressed a tissue-to-blood ratios. These two factors may balance one another at later time periods such that no significant differences could be observed from

3-72 hours. More importantly, these studies established that there may be two time ranges where this interaction is significant. ^{67}Ga-citrate biodistribution was influenced by cis-platinum shortly after the two agents were injected almost simultaneously on the fifth day of treatment, suggesting an immediate effect possibly due to a protein binding alteration. Tracer biodistribution was also changed after a much longer time frame which suggested the developing effects of the total drug dose were involved.

Attention was subsequently directed to further investigate these phases of the cis-platinum ^{67}Ga-citrate interaction.

B. In Vitro Protein Binding Analysis

Several experiments were planned to study the effects of cis-platinum and a related platinum compound (K_2PtCl_4) on the ability of transferrin and serum to bind ^{67}Ga-citrate, since any disturbance in this reaction could account for the observed early effects. Prior to an analysis of the results of these studies, a discussion on the methodology employed and the problems encountered will be presented.

1. Methodological Aspects

Several techniques were investigated in relation to their ability to efficiently separate the free from protein bound ^{67}Ga activity. A method was sought that satisfied several conditions including ease of analysis, minimum degree of non-specific binding and reproduceability. It was hoped that correlation between specific methods could be developed, such that results could be verified by another method. The suitability of the proposed methods was therefore

investigated.

Ultrafiltration: Comparison of the dry cone weight and the weight after a distilled water pre-soak followed by centrifugation showed that less than 30 µl of water remained in the cones at this time which represents a dead volume. The first ultrafiltrate of the test solution was discarded in order to further minimize errors associated with dilution of the ultrafiltrate with residual cone water. An inherent problem of small volume ultrafiltration is the determination of exact operating conditions. Only a small amount of ultrafiltrate (assumed to be free from dilution errors) should be collected so that the total volume inside the cone is not significantly affected. If a large volume is filtered, the protein concentration in the test solution is increased and since the degree of binding is concentration dependent, errors may occur. Usually, if less than 10% of the initial volume is collected, these errors can be neglected[628].

The cones were also checked for non-specific binding of ^{67}Ga formulations, with the results shown in Table 18. There is a wide variation among individual cones in terms of their ability to retain ^{67}Ga solution in the absence of protein. Even the same cone showed different values on separate occasions. This makes the accurate determination of control values very difficult. The high absolute binding of both $^{67}GaCl_3$ and ^{67}Ga-citrate in buffer to the cone components is a second deterrent to their use. Binding to added proteins must be sufficiently high to make this non-specific interaction insignificant. Others have since reported this problem of a high degree of uncertainty using this method and the cones were

generally used in situations where the expected bound activity would be high[629]. However, this method has the distinct advantage of a very short analysis time.

Table 18. Nonspecific binding of ^{67}Ga to ultrafiltration cones.+

^{67}Ga compound*	% ^{67}Ga bound to cone in absence of transferrin (mean ±s.d.; n=12)	range
$^{67}GaCl_3$	29 ± 6.1	18-50
^{67}Ga-citrate	24 ± 4.2	13-51

+ Amicon CF-25
* in buffered saline

Dialysis: The dialysis technique tested utilized a self-contained unit which allowed easy access to both compartments of the system and was readily amenable to procedures to aid the equilibration process such as shaking or rotating the sample units. Comparative analysis of ^{67}Ga bound to transferrin during several trial experiments is shown in Table 19. The bound fraction, as determined by G-50 gel chromatography was collected in each case and an aliquot was subjected to dialysis. The results show a drastic decrease in the percent bound as measured by dialysis. Similarly, it has also been found that when compared to ultrafiltration, dialysis tends to overestimate the free ^{67}Ga activity[384]. This is likely a consequence of dissociation of the ^{67}Ga-transferrin complex under the conditions of large volume dilution with a dialysing buffer for extended periods of time. Phosphate buffer was specifically avoided because it has been shown to cause almost complete removal of ^{67}Ga from its

transferrin binding sites during dialysis procedures[386].

The temperature at which the system is equilibrated also affects the observed degree of protein binding. Dialysis performed in the cold (4°C) showed a significantly greater percent bound (35%) compared to those systems equilibrated at physiological temperature (2%)[629].

Gel chromatography: The principles of gel chromatography have been fully explained in relation to their ability to separate macromolecules from low molecular weight components, and thus, protein bound and free ^{67}Ga could be easily separated using such a system.

Artifacts due to the separation of free ^{67}Ga from serum proteins by this method have also been reported. Generally when serum samples are analyzed by both dialysis and gel chromatography, a significantly higher degree of binding was measured by dialysis[377], which is supported by the data in Table 19. In addition, when the bound fraction of an initial gel chromatographic separation was passed through the column a second time less than half the original percent binding was observed[363]. Both of these results have been attributed to an interaction of the gel with the ^{67}Ga-transferrin complex causing dissociation. This may also be related to a buffer interaction or dilution effect observed for the dialysed samples since a similar pattern of reduced binding subsequent to an initial separation is shown in Table 19. This type of explanation would account for the apparently anomalous results shown for both dialysis and gel chromatography.

The relative ease of sample analysis by the gel chromatography system utilizing the almost totally automatic separation system described earlier made this method very convenient.

Thus, it was found that ultrafiltration of samples could not be reliably used to determine the extent of binding of ^{67}Ga to transferrin, even though the inherent problems could be overcome. Both gel chromatography and dialysis could be reliably used in themselves as a relative measure of binding although absolute determinations could not be obtained.

Table 19. Comparison of ^{67}Ga binding to transferrin as measured by gel chromatography and dialysis.

Sample	% Bound as determined by Gel Chromatography*	Dialysis+
1	> 98	10.7
2	> 98	10.7
3	> 98	10.3

* Collection of bound fraction
\+ Bound fraction of gel chromatography subjected to dialysis for 24 hours

Sample	% Bound as determined by Dialysis+	Gel Chromatography*
A	85.5	32.2
B	82.3	31.9
C	89.6	38.8

\+ Calculated from observed degree of protein binding by dialysis method
* Gel chromatography of dialysed sample

2. Effects of Platinum Compounds on the Binding of ^{67}Ga-citrate to Transferrin and Whole Sera in vitro.

The possible effects of the presence of either of two platinum compounds on the observed association of ^{67}Ga to transferrin was studied by means of an in vitro incubation system. Both dialysis and gel chromatography were utilized as separation techniques for individual experiments. The experimental protocol in the dialysis study was designed to observe the magnitude of this interaction after a short incubation period and with a high platinum to transferrin ratio in the solution. In subsequent gel chromatography experiments, this design was expanded to include different anion systems with analysis of both ^{67}Ga and platinum binding over extended periods. There is no apparent effect of cis-platinum on ^{67}Ga binding over time periods up to 90 minutes at low platinum to transferrin ratios[630].

The incubation of ^{67}Ga and platinum reagents with an excess of one of the anion solutions allows for the possibility of metal-complex formation and subsequent binding to transferrin in the specific metal binding sites. It has been shown that many metal ions require these anions for specific binding to occur. For example, in the absence of these agents ferric salts bind non-specifically and tightly to transferrin and only slowly move to the specific sites[631]. NTA was chosen as a representative of the highly effective in vitro agents while the citrate/bicarbonate system would be closely related to the in vivo situation.

The chloride ion concentration and pH of the buffer were adjusted to near physiological plasma levels to approximate in vivo conditions.

The reactivity of these platinum compounds in aqueous buffered solutions is complicated by the formation of numerous aquated and hydroxo species, while ^{67}Ga-chloride may be expected to form various hydroxides, both of which will influence the rate and extent of the cumulative binding process. In addition, buffer competitive effects may also play a role in the observed association of ^{67}Ga and both platinum compounds with transferrin[632].

The reagent concentrations and other conditions for the dialysis study are summarized in Table 7. The results of ^{67}Ga binding to transferrin compared to its binding in the presence of cis-platinum or K_2PtCl_4 is shown in Figure 12, and are expressed as the comparative percent bound. It is apparent that K_2PtCl_4 significantly reduces ^{67}Ga binding to transferrin whereas in the presence of cis-platinum no significant difference was observed. The magnitude of the K_2PtCl_4 effect is an approximate 30% reduction in ^{67}Ga binding during the 24 hour incubation period at an initial platinum to transferrin ratio of 200 to 1. These initial results of a platinum induced decrease in ^{67}Ga association with transferrin initiated further studies of this effect.

The gel chromatographic experiments utilized similar conditions (Table 7) and a second anion system with additional analysis of platinum binding. Both cis-platinum and K_2PtCl_4 are observed to associate with transferrin in the presence of either the citrate/bicarbonate or the NTA anions (Figure 13). Cis-platinum was bound 3-4 times less than K_2PtCl_4 which is reflected in the percent bound value. No significant differences were observed for each compound with respect to a preference for one of the anion systems,

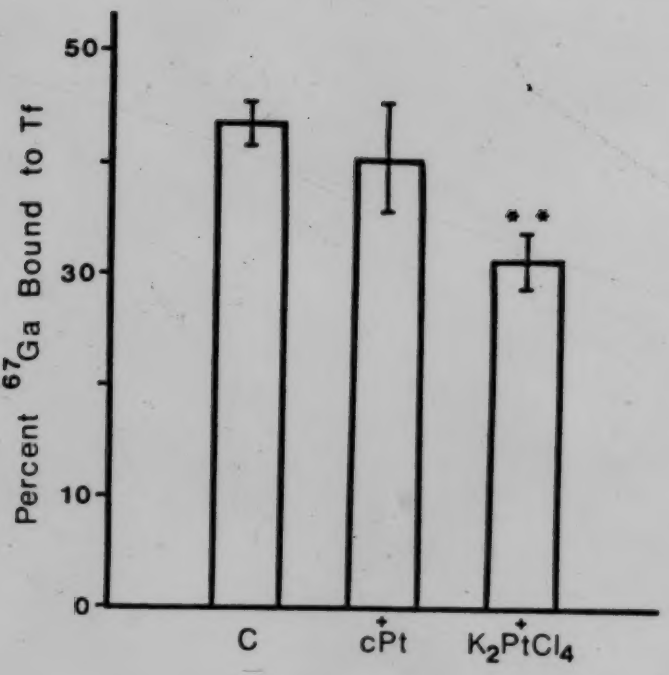


Figure 12. Relative binding of Ga-67 to transferrin in buffered saline by the Ga-67 citrate control (C) and with the addition of cis-platinum (cPt) and K_2PtCl_4 . Separation by dialysis. (mean ± s.d. ; **$p < 0.01$; n=5)

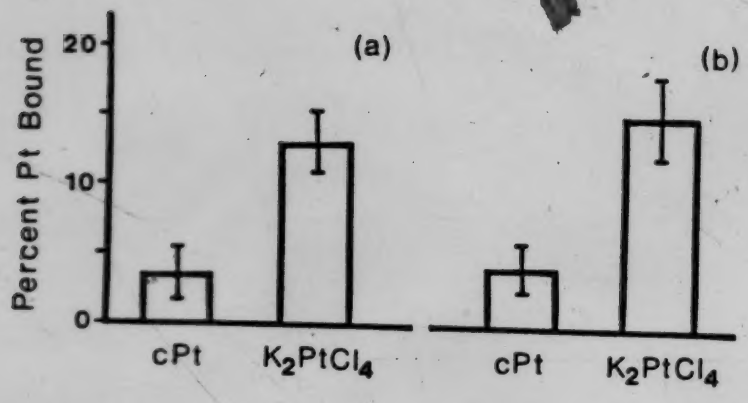


Figure 13. Relative binding of platinum from cis-platinum (cPt) and K_2PtCl_4 in buffered saline in the presence of (a) NTA anions and (b) HCO_3^-/citrate anions. Separation by gel chromatography. (mean ± s.d.)

even though anion concentrations varied greatly between the two systems. This may be related to a lack of platinum-anion interaction such that the protein binding would be independent of the anion and non-specific in nature. ^{67}Ga binding showed a definite preference for the citrate/bicarbonate system with an increase by a factor of two when compared to the case where NTA only was present. Bicarbonate has been shown to be a more efficient cofactor than NTA in terms of its ability to act synergistically in the cation binding process[633], although it may also be related to the concentration of the anions.

The effect of cis-platinum and K_2PtCl_4 on the observed ^{67}Ga binding is presented in an analogous manner to the dialysis results in Figure 14. A significant difference between controls and platinum containing incubations was observed in both anion systems. This decrease was more significant in the case of the NTA anion and for K_2PtCl_4 compared to cis-platinum. The reduction of ^{67}Ga binding to transferrin relative to control reflects these differences (Figure 14a).

The best comparison of the effects of platinum binding on the association of ^{67}Ga with transferrin is shown in Figure 15, where the calculated number of platinum atoms bound per transferrin atom is plotted versus the observed percent of ^{67}Ga binding relative to controls. These graphs suggest a relationship between the data such that as platinum binding increases the relative ^{67}Ga binding decreases. At about four bound platinum molecules per transferrin ^{67}Ga binding is reduced to ~40% (Figure 15) and if one assumes one platinum molecule displaces one ^{67}Ga atom then the number of ^{67}Ga atoms bound in the absence of platinum would be 10 ^{67}Ga atoms per

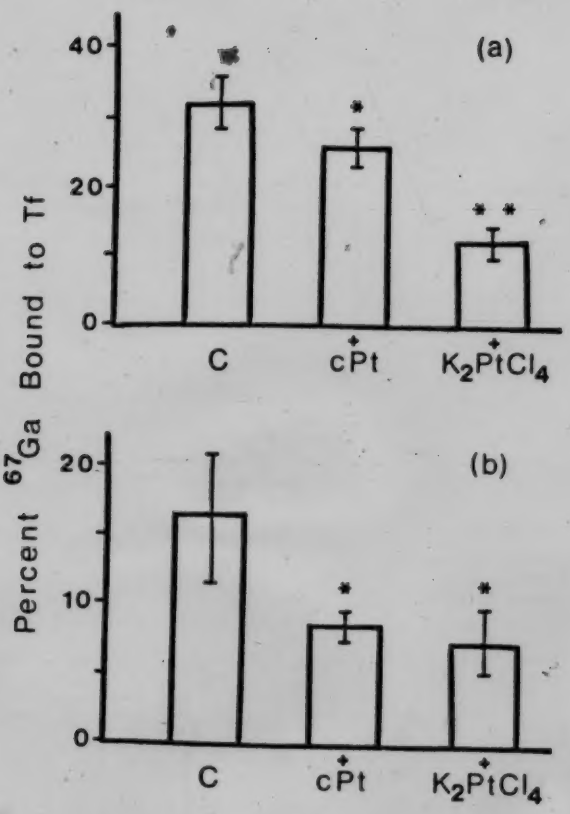


Figure 14. Relative binding of Ga-67 to transferrin in buffered saline by the Ga-67 citrate control (C) and with the addition of cis-platinum (cPt) and K_2PtCl_4 in the presence of various anions. (a) NTA anions ; (b) HCO_3^- /citrate anions. Separation by gel chromatography. (mean ± s.d. ; * $p < 0.05$; ** $p < 0.01$; n=5)

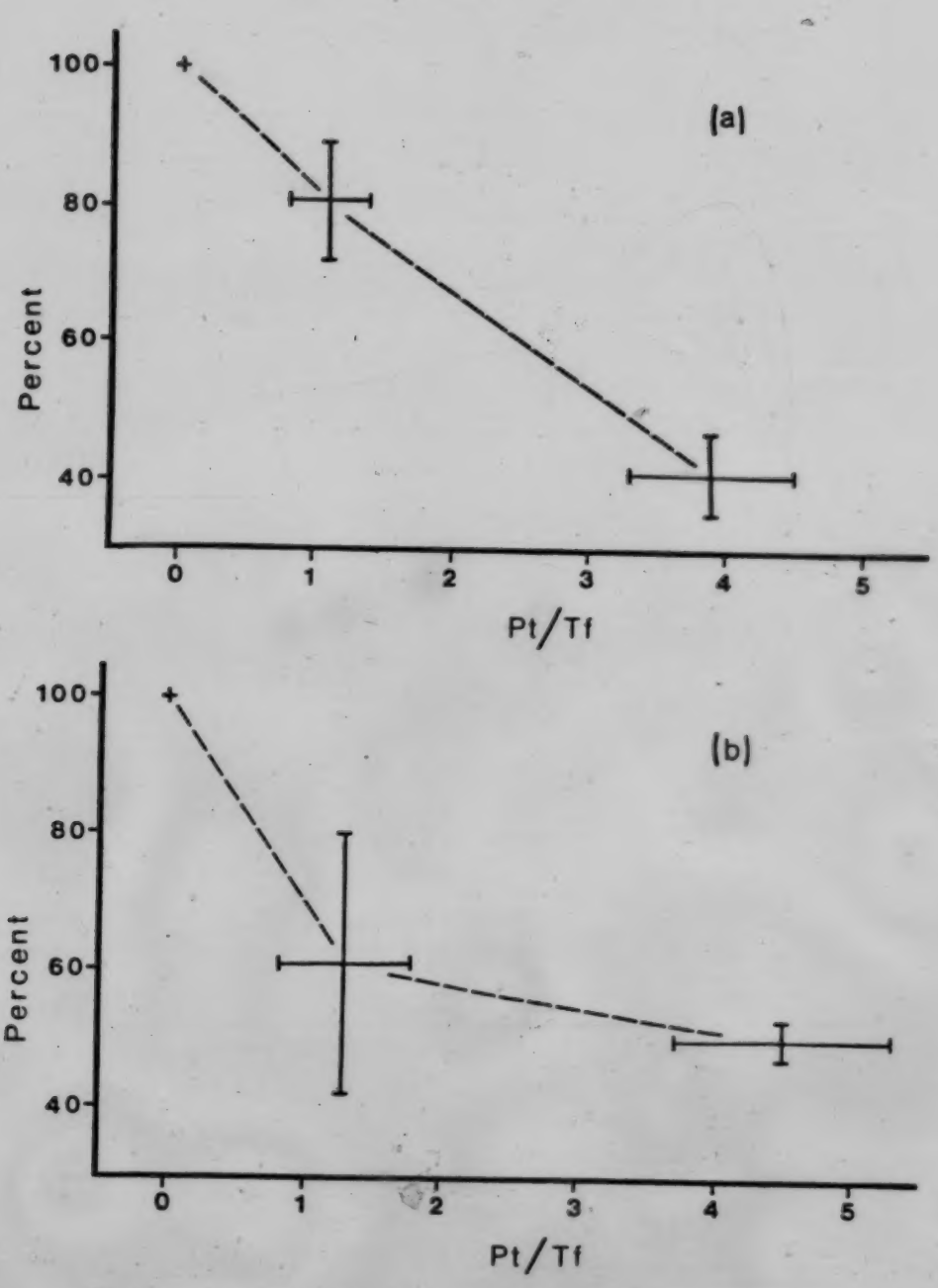


Figure 15. Relationship between the calculated number of platinum atoms bound per transferrin molecule (from both cis-platinum and K_2PtCl_4) and the percent binding of Ga-67 relative to controls. Conducted in buffered saline in the presence of (a) NTA anions and (b) HCO_3^-/ citrate anions. Separation by gel chromatography. (mean ± s.d. of each determination)

transferrin molecule. It has been reported that transferrin has 14 binding sites per molecule for colloidal gallium citrate based on thermodynamic calculations[381], while conductometric studies indicate about 20 binding sites for the hydrated Ga(III) ion[405].

The tetrachloroplatinite anion ($PtCl_4^{-2}$) is generally bound to the periphery of proteins with sulphur groups and nitrogen bases being the main centres of attachment[634], and we would expect a similar pattern for cis-platinum. Since the anion is charged, the observed increased binding of K_2PtCl_4 may be due to additional electrostatic interactions or to the availability of more labile ligands compared to cis-platinum.

A wide variety of metal cations have been shown to bind specifically to human transferrin, including most of the period 4 metals, as well as several of the lanthanide and actinide series[17]. The binding of heavy metals of Group VIII of the periodic table, such as platinum have not been investigated in detail. The results presented here suggest the association of cis-platinum with transferrin may be nonspecific in nature under these conditions since the saturation value of two platinum atoms bound per protein molecule is not observed. This may be due to some residual iron in the specific sites or because the strongly complexed ammine ligands preclude such an association. In the case of K_2PtCl_4, specific and non-specific binding may be occurring because more than two atoms of platinum were observed to bind per transferrin molecule. In light of the preferred attachment sites and reactivity of this molecule, it seems unlikely it would preferentially seek out the tyrosine ligands which are implicated as the specific metal binding ligands of

transferrin. However, in both cases a reduction in the binding of ^{67}Ga was observed such that if specific binding was assumed for ^{67}Ga then some degree of specific binding of both platinum compounds could be concluded. A claim of specific platinum binding to transferrin has been reported for K_2PtCl_4 in a similar *in vitro* study[635]. Alternately, the platinum could be affecting the conformation of the specific binding sites causing a physical hindrance. Also, since it appears ^{67}Ga is bound to more than just the specific sites, the platinum may be displacing it from these sites.

In light of these results with isolated transferrin solutions, we might expect cis-platinum to similarly affect ^{67}Ga-citrate binding in whole sera. However, the data in Table 20 indicates this is not the case, in spite of the fact that the concentrations of platinum reagents used were several orders of magnitude greater than those expected *in vivo*. Radiolabeled cis-platinum studies under identical conditions showed that by 72 hours 60-70% of cis-platinum was bound yet at no time was interference with ^{67}Ga-citrate binding observed. Even when either cis-platinum or K_2PtCl_4 was pre-incubated with serum for 24 hours, which would maximize platinum binding, no decrease in ^{67}Ga-citrate binding could be detected. Cis-platinum is bound to a variety of proteins including globulin, albumin and transferrin in serum[50,51,53] but albumin accounts for the greatest majority[60]. The reactivity of both platinum compounds suggests no specific binding to transferrin takes place when other high affinity or high capacity proteins such as albumin are present. Thus although the drug is capable of binding to human transferrin, this appears to be of a non-specific nature with no preference for this protein *in vivo*. Any

in vivo modifications of ^{67}Ga protein binding must be due to another mechanism rather than direct displacement.

Table 20. Effect of cis-platinum and K_2PtCl_4 on the *in vitro* serum binding of ^{67}Ga-citrate.

Incubation duration (hours)	Duration of dialysis* (hours)	% ^{67}Ga bound relative to control† (mean ± s.d.; n=3)		% cis-platinum bound** (mean ± s.d.; n=3)
0		+ cis-Pt	+K_2PtCl_4	
	16	102.2 ± 4.7	-	-
	24	-	-	32.0 ± 1.7
	48	99.5 ±3.9	-	48.4 ± 10.4
	60	101.1 ± 5.4	-	-
	72	-	-	66.4 ± 6.1
24				
	24	104.2 ± 5.4	98.9 ± 3.2	-
	48	101.1 ± 2.9	99.2 ± 3.3	-
	118	101.6 ± 4.9	105.2 ± 4.8	-

* In addition to incubation duration

† ^{67}Ga bound with platinum/^{67}Ga bound without platinum x 100

** radiolabeled cis-platinum prepared by direct activation

C. Mice Studies

1. Drug Combination Study

Cis-platinum is rarely administered as a single agent and, in fact, the initial clinical observations of unusual ^{67}Ga-citrate images after cis-platinum treatment were made in patients who were also receiving vinblastine, bleomycin and doxorubicin. Although the possibility that cis-platinum was largely responsible for the altered biodistribution of the tracer prompted this work, a study was conducted with mice to evaluate the effects of the total drug combination on ^{67}Ga-citrate localization before proceeding to other animal investigations.

The doses of each agent employed was based on the standard testicular cancer regimen employed in stage 3 non-seminoma patients. Only the single daily dose for each drug was used in cases where the drugs would normally be given several consecutive days. Human doses were scaled down appropriately for the mouse model based on the relative body surface areas of the two species, which has been shown to adequately approximate an equivalent toxic response[636].

The effect of such combinations on the 3 hour distribution of ^{67}Ga-citrate is shown in Figure 16. At this time, there is a significant decrease in the ^{67}Ga localization in bone, tumor, stomach, large intestine, heart and blood and a significant increase in the kidney uptake for both the 3 drug [vinblastine, adriamycin and bleomycin (VAB)] and the 4 drug [vinblastine, adriamycin, bleomycin and cis-platinum (VAB cPt)] combinations. Cis-platinum treatment alone showed a significant but less dramatic decrease only in bone and large intestine. This general lack of effect at this time period

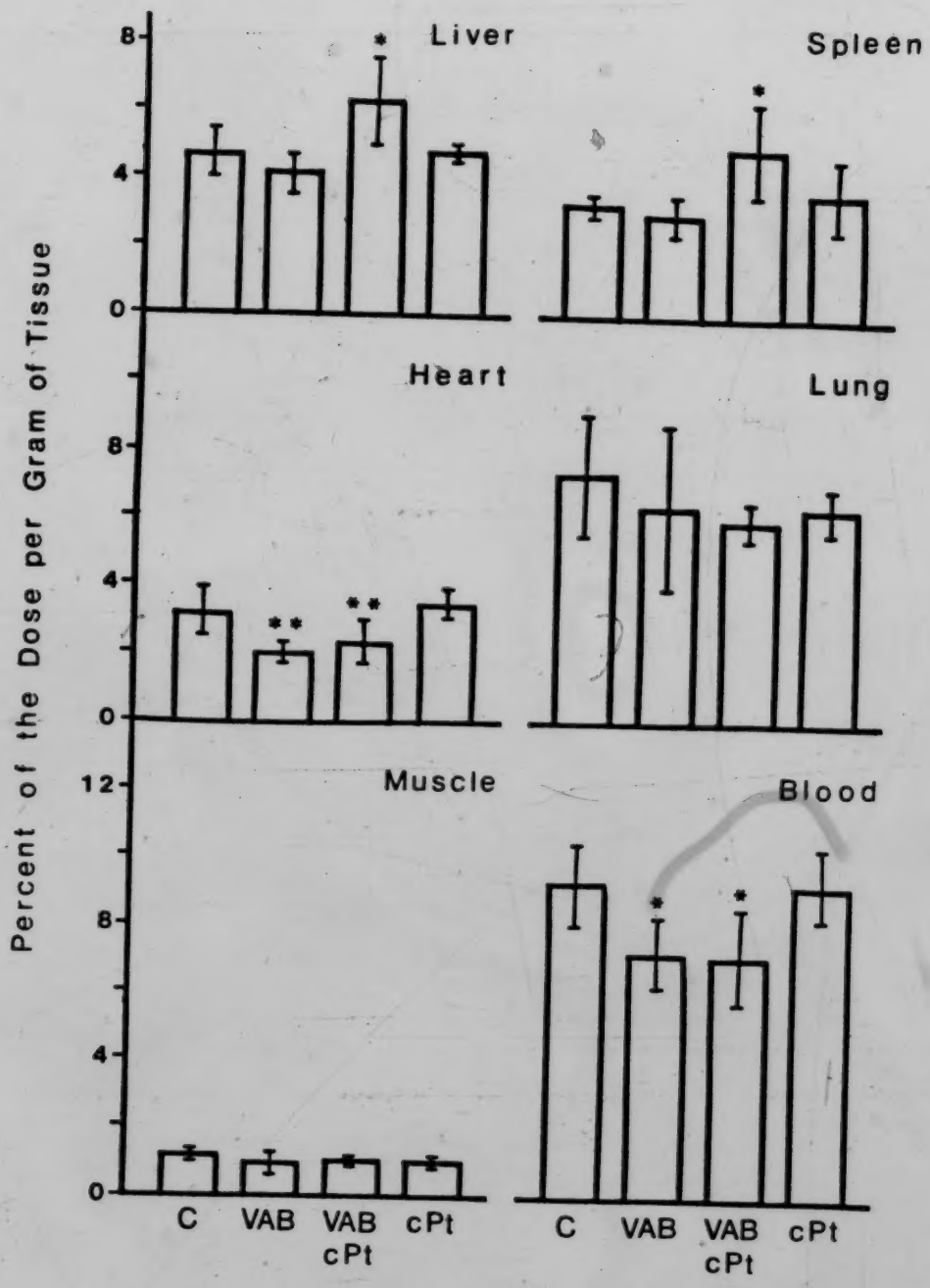


Figure 16a. Study of the effects of Vinblastine, Adriamycin, and Bleomycin (VAB) ; Vinblastine, Adriamycin, Bleomycin, and cis-platinum (VAB cPt) ; and cis-platinum (cPt) treatment on the 3 hour tissue distribution of Ga-67 citrate in RI lymphoma bearing CBA/CAJ mice compared to contols (C). (mean ± s.d. ; * $p < 0.05$; ** $p < 0.01$; n=4-6)

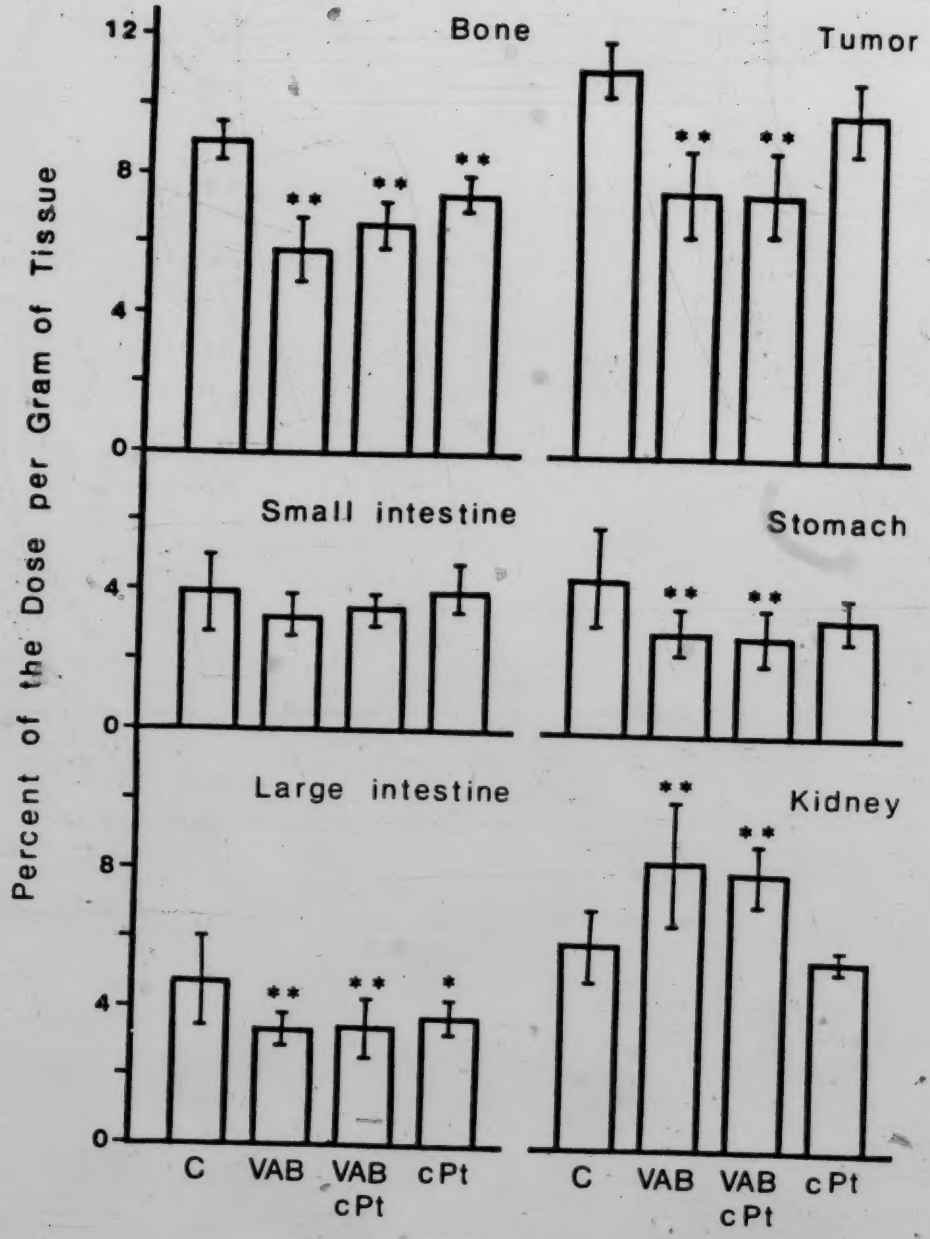


Figure 16b. Study of the effects of Vinblastine, Adriamycin, and Bleomycin (VAB) ; Vinblastine, Adriamycin, Bleomycin, and cis-platinum (VAB cPt) ; and cis-platinum (cPt) treatment on the 3 hour tissue distribution of Ga-67 citrate in RI lymphoma bearing CBA/CAJ mice compared to controls (C). (mean ± s.d. ; $*p < 0.05$; $**p < 0.01$; n=4-6)

supports the conclusion of the binding studies that acute dosing should not drastically affect the short term distribution of ^{67}Ga-citrate. In the VAB and VAB cPt treated groups, the noticeable increase in kidney levels of the tracer indicate the possible induction of rapid diuresis or an inflammatory response which made less tracer available for tissue incorporation and generally reduced all normal tissue uptake processes.

By 48 hours after drugs and tracer injection, only stomach, small intestine and blood continue to show significantly less uptake in the VAB and VAB cPt treated groups, while only blood levels were reduced in the cPt treated animals (Figure 17). The gastrointestinal effects at 48 hours in the drug combination treated groups are likely related to local irritation and denudation caused by these agents, the physical results of which were observed in these animals. Reduced blood levels in these groups are likely related to the early effects, but were not expected in the cis-platinum group.

This study serves to illustrate that the immediate effects of VAB combination drug treatment are substantially different from those of cis-platinum and that acute dosing results in only slight changes in ^{67}Ga-citrate localization after longer distribution phases. Although the individual drugs given as single therapeutic doses may alter ^{67}Ga-citrate localization, the ability of cis-platinum to produce characteristic effects after cumulative doses may present a similar model by which many toxic chemotherapeutic agents act in altering the physiological handling of ^{67}Ga-citrate.

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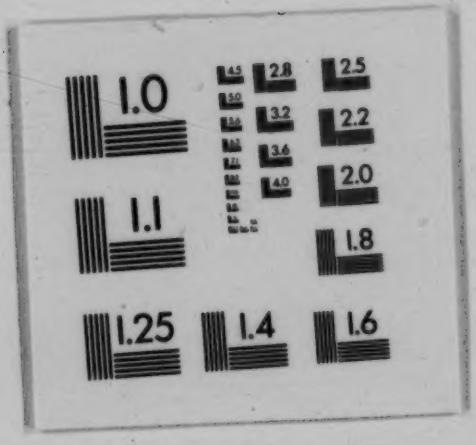


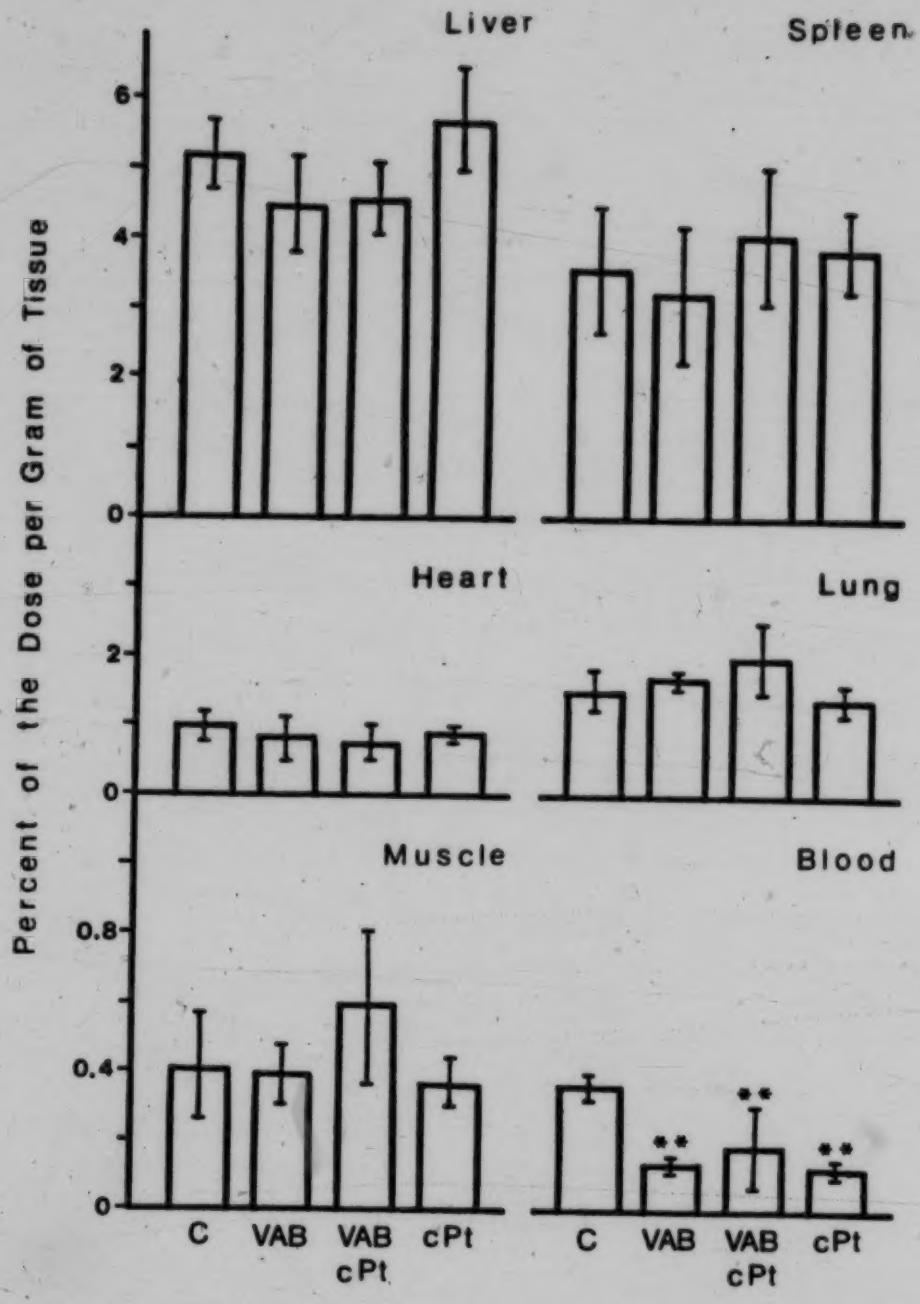


Figure 17a. Study of the effects of Vinblastine, Adriamycin, and Bleomycin (VAB) ; Vinblastine, Adriamycin, Bleomycin, and cis-platinum (VAB cPt) ; and cis-platinum (cPt) treatment on the 48 hour tissue distribution of Ga-67 citrate in RI lymphoma bearing CBA/CAJ mice compared to controls (C). (mean ± s.d. ; $*p < 0.05$; $**p < 0.01$; n=4-6)

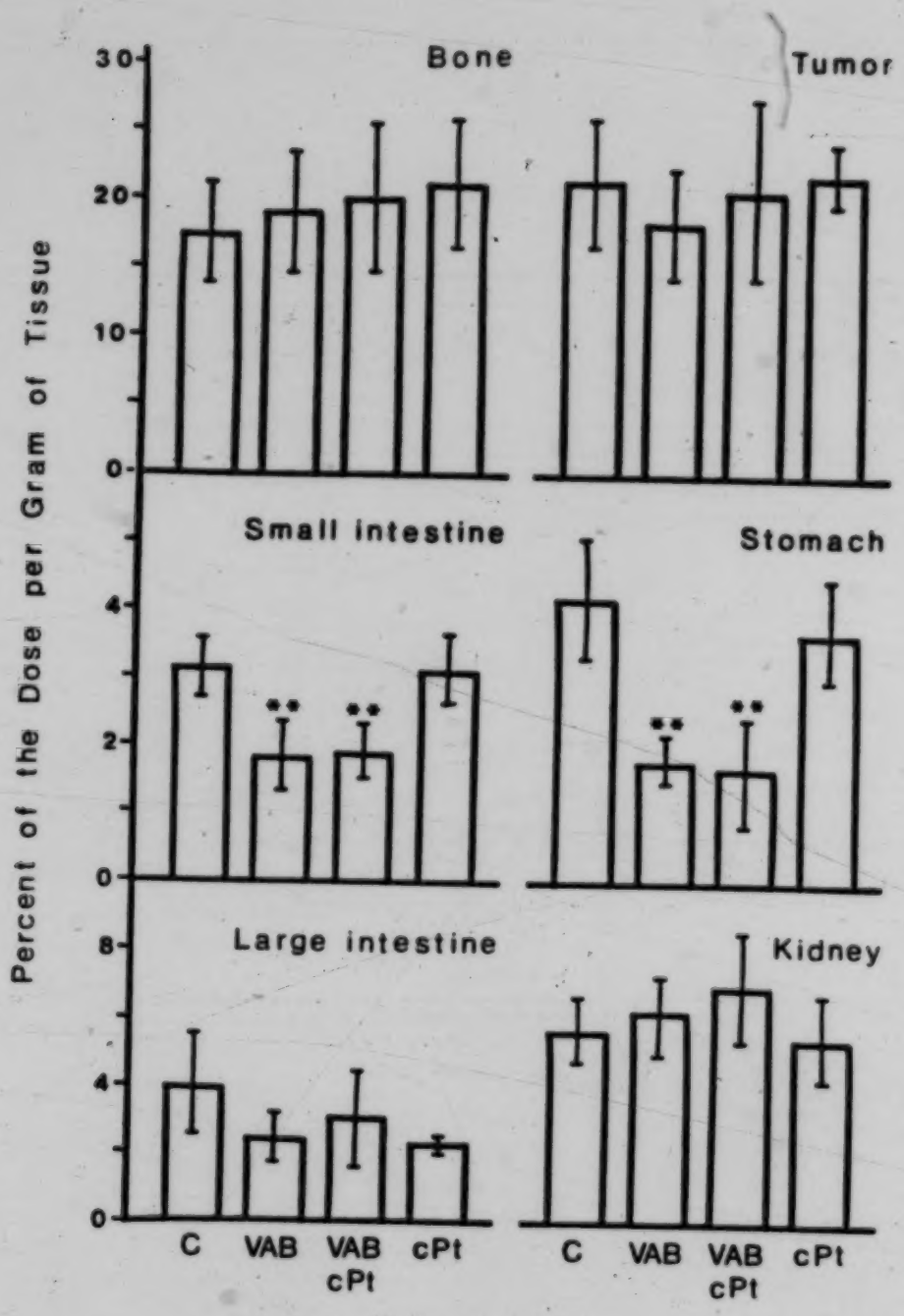


Figure 17b. Study of the effects of Vinblastine, Adriamycin, and Bleomycin (VAB) ; Vinblastine, Adriamycin, Bleomycin, and cis-platinum (VAB cPt) ; and cis-platinum (cPt) treatment on the 48 hour tissue distribution of Ga-67 citrate in RI lymphoma bearing CBA/CAJ mice compared to controls (C). (mean ± s.d. ; $*p < 0.05$; $**p < 0.01$; n=4-6)

2. Study of the Immediate and Delayed Effects of cis-Platinum Treatment on the Short Term Biodistribution of ^{67}Ga-citrate.

The protein binding experiments established that although cis-platinum slowly reacts with human transferrin in solution, it does not affect ^{67}Ga-citrate binding to normal human sera *in vitro*. Thus, an acute dose of cis-platinum would not be expected to alter the short term biodistribution of ^{67}Ga-citrate via a direct interaction. However, cumulative doses of the drug and its consequences may be responsible for the short term change that was evident in the initial study in rats. This possibility was tested using the mouse model and a specific drug administration protocol. Each group of treated mice received 5 mg/kg of cis-platinum by intravenous injection with ^{67}Ga-citrate and some (10,15 and 20 mg/kg cumulative dose groups) previously received additional 5 mg/kg intraperitoneal injections of cis-platinum (one, two and three respectively) at two day intervals, resulting in successively higher cumulative doses. The short term (3 hours) distribution of ^{67}Ga-citrate after such a regimen is shown in Figure 18. All the tissues examined, with the exception of liver and spleen, show no changes in uptake after the co-administration of cis-platinum. However, after at least one pretreatment with the drug an increased accumulation of the tracer was noted in virtually all tissues. Higher cumulative cis-platinum generally results in greater uptake but it is apparently not a strict function of dose. Another report dealing with the effects of cis-platinum on the biodistribution of ^{67}Ga-citrate in mice are difficult to compare to these results due to differences in the injection schedule. However, in the most comparable situation, it was observed that blood, spleen and liver

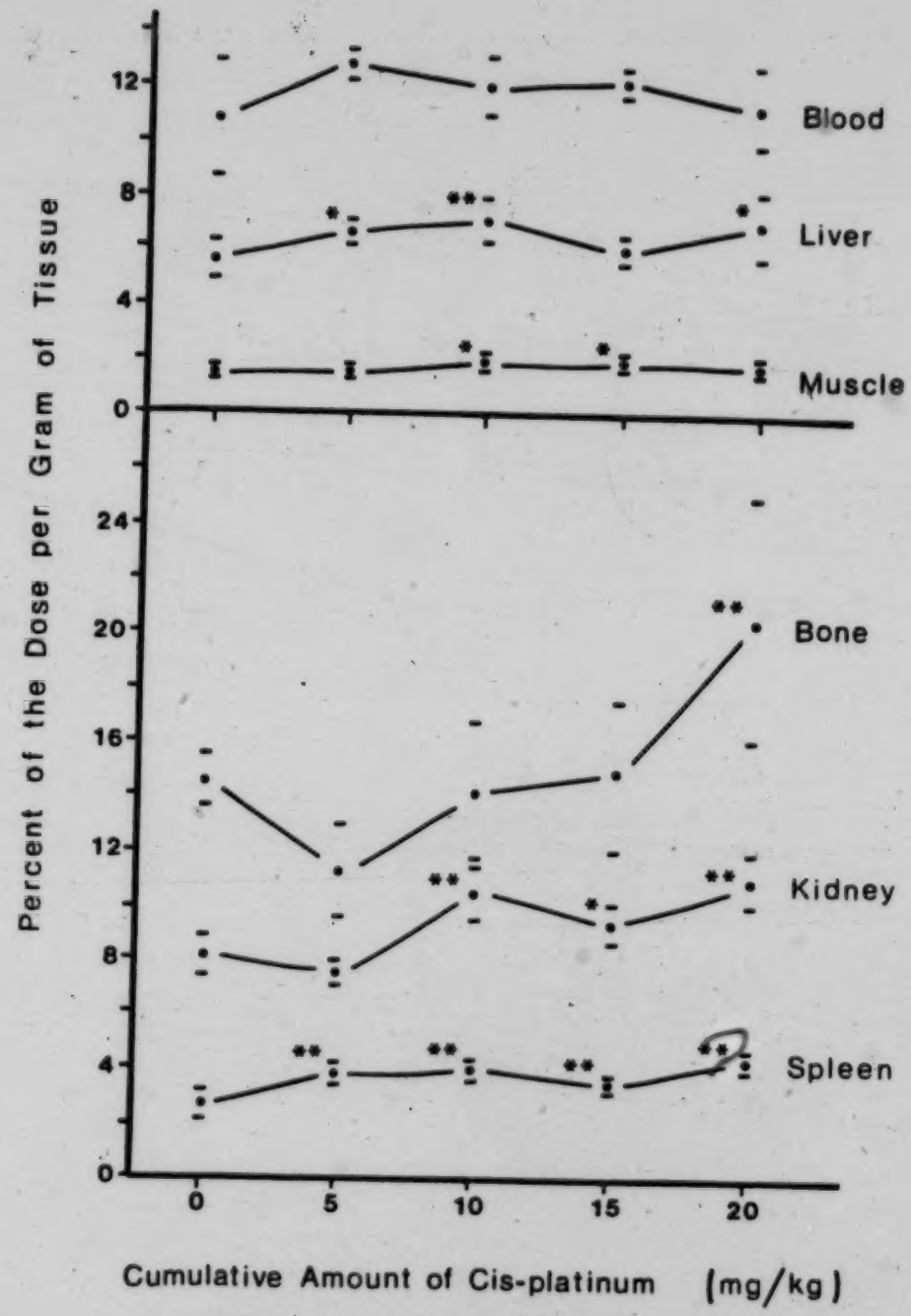


Figure 18a. Study of the immediate (5 mg/kg) and accumulated (5 mg/kg at 2 day intervals) effects of cis-platinum treatment on the 3 hour tissue distribution of Ga-67 citrate in CBA/CAJ mice relative to the control group (0 mg/kg). (mean ± s.d. ; * $p < 0.05$; ** $p < 0.01$; n=4-6)

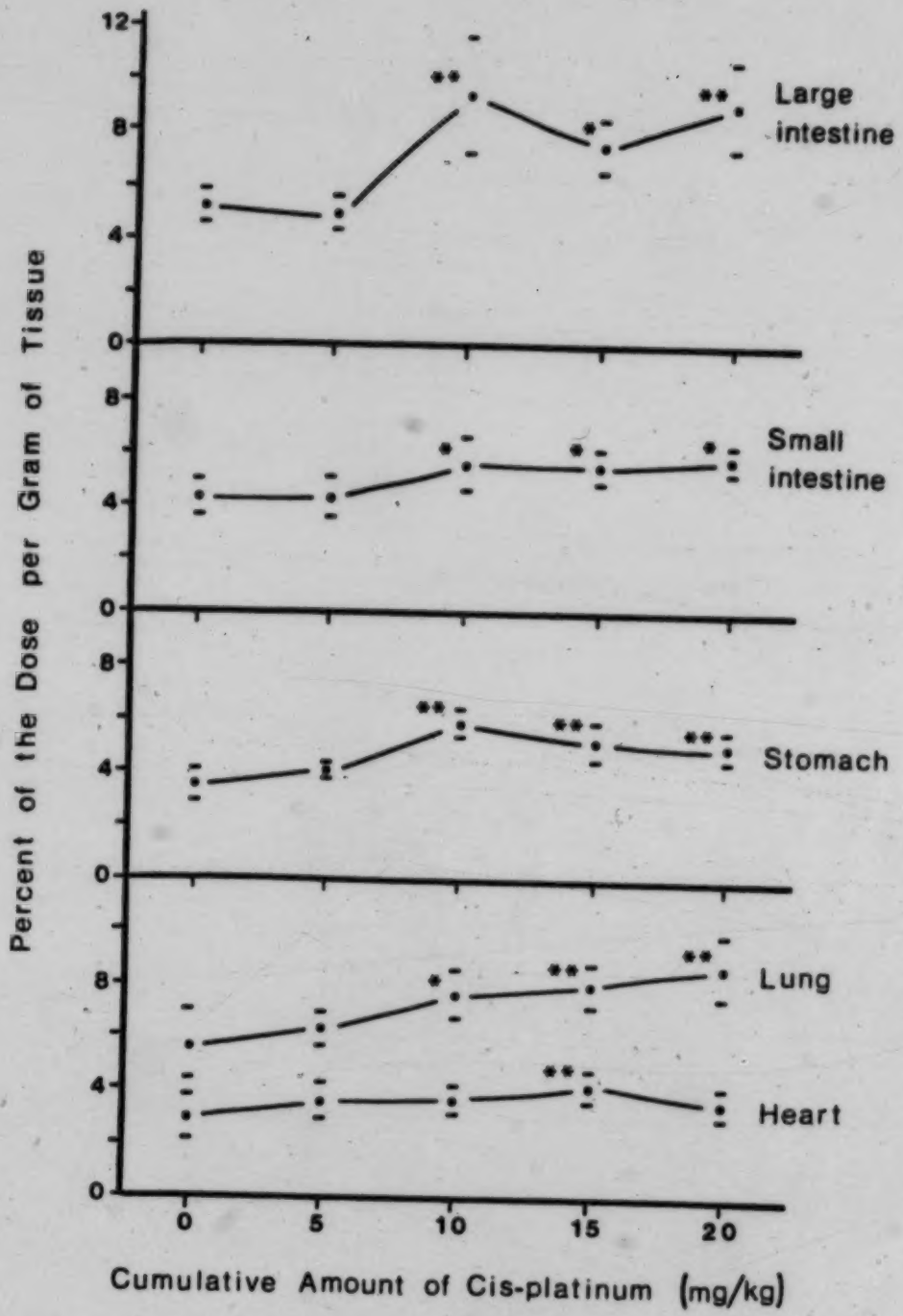


Figure 18b. Study of the immediate (5 mg/kg) and accumulated (5 mg/kg at 2 day intervals) effects of cis-platinum treatment on the 3 hour tissue distribution of Ga-67 citrate in CBA/CAJ mice relative to the control group (0 mg/kg).
(mean ± s.d. ; * $p < 0.05$; ** $p < 0.01$; n=4-6)

levels were reduced after cis-platinum treatment[637]. This is in contrast to the results presented here and may be related to the inclusion of mannitol in the injection of cis-platinum used in the other studies, which lessens the overall toxicity of the drug, and may hasten the elimination of subsequently administered ^{67}Ga-citrate. The cumulative dosing schedule also allows the development of cis-platinum toxicities, which may include anorexia. This factor has previously been shown to increase tissue levels of ^{67}Ga in liver, spleen, bone marrow and kidneys[486] as was demonstrated here.

Blood levels are not significantly different from control values and thus we would expect increased tissue uptake to be reflected as an increased tissue-to-blood ratio. These data are presented in Table 21. Again, the lack of immediate effects of cis-platinum on ^{67}Ga-citrate localization can be observed as well as a general increase in tissue-to-blood ratios after at least one drug pretreatment. Cases where no significant difference between control and treated groups was apparent in these data compared to the percent of the dose per gram data indicate slight changes in blood values can offset tissue concentration changes and that the larger error and small number statistics associated with these figures make interpretation more difficult. However, in most cases (75%) the relative tissue uptake pattern was the same for both methods of data handling.

These results offer <u>in vivo</u> support for the serum binding studies and substantiate that the early effect of cis-platinum on ^{67}Ga-citrate localization observed in the rat study was due to previously injected drug doses. In light of the nonspecific increase

in tissue uptake of the tracer, it is likely due to a cis-platinum induced renal toxicity, which takes 2 - 3 days to develop[303], and which may be preventing normal urinary excretion and allowing greater tissue accumulation to proceed.

The three hour distribution of cis-platinum after a single 5 mg/kg dose in a similar group of mice was assessed using the radiolabeled compound. As shown in Table 22, the compound shows no large preference for any tissue at this time, although the highest levels are observed in the kidney, bone and liver with relatively little in the muscle. Thus, the drug is present to some degree in all tissues at this time and its lack of effect on ^{67}Ga-citrate distribution after a co-administered dose cannot be argued on the basis of specific localization. However, the biochemical consequences of such deposition may ultimately be responsible for individual tissue changes in ^{67}Ga uptake.

Table 21. Effect of cumulative amounts of cis-platinum on the three hour tissue-to-blood ratios (% dose per gram tissue/% dose per gram blood) of ^{67}Ga in CBA/CAJ mice.

	Cumulative amount of cis-platinum (mg/kg)					
Tissue	0	5	10	15	20	Level of sig *
heart	0.28±0.03	0.28±0.04	0.31±0.03	††0.34±0.04	††0.33±0.03	p<0.005
lungs	0.52±0.03	0.50±0.04	0.63±0.10	††0.66±0.06	††0.82±0.09	p<0.005
liver	0.52±0.04	0.52±0.02	††0.62±0.04	0.48±0.03	††0.65±0.04	p<0.005
blood	1.00	1.00	1.00	1.00	1.00	-
stomach	0.31±0.01	0.31±0.03	††0.49±0.07	††0.42±0.06	††0.51±0.08	p<0.005
spleen	0.26±0.04	0.30±0.02	††0.34±0.01	0.29±0.01	††0.42±0.07	p<0.005
muscle	0.15±0.02	0.12±0.15	0.16±0.02	0.16±0.03	0.17±0.04	N.S.
Small Intestine	0.40±0.04	0.33±0.06	0.46±0.05	0.44±0.05	††0.54±0.12	p<0.005
Large Intestine	0.49±0.07	0.39±0.06	††0.80±0.21	0.60±0.08	††0.86±0.25	p<0.005
kidney	0.77±0.08	0.60±0.03	†0.91±0.07	0.76±0.07	††1.03±0.18	p<0.005
bone	1.38±0.03	0.89±0.14	1.21±0.37	1.13±0.18	††2.02±0.74	p<0.005

*ANOVA n=4-6
†significantly different from control p<0.05 LSD test
††significantly different from control p<0.005 LSD test

Table 22. Tissue distribution† of radiolabeled cis-platinum* in CBA/CAJ mice three hours after intravenous injection.

heart	lungs	liver	blood	stomach
1.90 ±0.19	2.23 ± 0.25	2.74 ± 0.78	1.52 ± 0.34	1.45 ± 0.39
spleen	**muscle**	**gut**	**kidney**	**bone**
1.83 ± 0.56	0.59 ± 0.08	1.17 ± 0.21	3.62 ± 0.94	2.37 ± 0.52

† % dose per gram of tissue; n=4
* prepared by direct activation method

D. Effect of cis-Platinum Treatment on the Biological Behavior of ^{67}Ga-citrate in New Zealand White Rabbits.

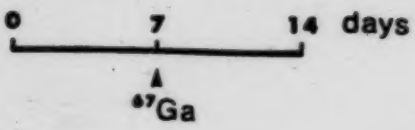
1. Study Design

New Zealand White rabbits were chosen as the biological model for a more comprehensive study of the effects of cis-platinum treatment on the in vivo behavior of ^{67}Ga-citrate. These animals were routinely available, readily housed and cared for and generally easily handled. They are large enough to allow for repetitive blood sampling and quantitative gamma camera imaging, such that a more complete assessment of individual animal status and ^{67}Ga behavior could be accomplished. The time schedule for the measurement of several aspects of the behavior of ^{67}Ga in rabbits and some other pertinent parameters is shown in Figure 19.

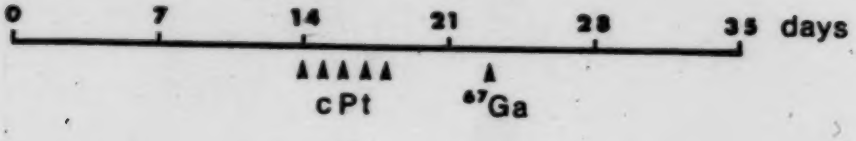
The dosage and treatment schedule of cis-platinum in this study is comparable to a common human regimen - 20 mg/m^2 daily for five days. This translates to about 0.5 mg/kg in humans for five days and represents a low to medium toxic dose in dogs[254]. Unlike the human protocol, which calls for combinations of hydration and diuretics, these animals received no adjuncts.

^{67}Ga-citrate was administered to treated animals five days after completion of this dosage schedule. During this period, some of the slow developing toxic reactions to the drug may be manifested and could be related to possible alterations in ^{67}Ga-citrate behavior. Earlier studies in vivo indicated the acute administration of the drug had no effect on tracer biodistribution unless dosing was separated by some period of time. In addition, this delayed administration of ^{67}Ga-citrate would more closely parallel the expected clinical

A. Control phase



B. Cis-platinum treatment phase



Ga-67 Behavior	Time of measurement during phase: A.	B.
Serum protein binding	7,9	23,26
Blood clearance	7-14	23-30
Whole body elimination	7-14	23-30
Urinary excretion	7-14	23-30
Fecal excretion	7-14	23-30
Urinary disposition	.8	24
Whole body localization		
-gamma camera imaging	7,8,9	23,24,25
-tissue sampling	9	25
Related parameters		
Serum creatinine	5	9,13,21
Serum iron and TIBC	5	9,13,21
Hematocrit	-	9,13,21,29
Food and water consumption	-	1-30
Body weight	-	9,14,16,19,21,24
Kidney pathology	9	25,34

Figure 19. Time schedule for the measurement of Ga-67 biological behavior and related parameters in rabbits.

situation where patients would be more likely to receive both agents in a time frame similar to the one used in these studies.

2. Serum Protein Binding

The ability of ^{67}Ga-citrate to bind to serum proteins *in vivo* and *in vitro* was investigated using both the dialysis and gel chromatographic techniques for the separation of free and bound ^{67}Ga fractions.

The *in vivo* binding was studied by blood sampling at 3 and 48 hours after ^{67}Ga-citrate administration to a group of control and cis-platinum treated rabbits. The results are shown in Table 23. There are no significant differences in the serum protein binding between the two groups at 3 or 48 hours, measured by either technique. There is a wide variation in the dialysis determinations, and a small range for the gel chromatographic measurements, such that in both cases group differences cannot be identified.

Table 23. Protein binding of ^{67}Ga in serum obtained 3 and 48 hours after administration of ^{67}Ga-citrate to control and cis-platinum treated rabbits.

	Percent Bound		
	mean ± s.d.	(n)	range
A. Dialysis results (3 hours)			
control rabbits	15.8 ± 7.2	(3)	7.9 - 22.1
cis-platinum treated rabbits	29.8 ± 25.3	(4)	10.3 - 66.1
B. Gel chromatographic results (3 hours)			
control rabbits	97.1 ± 1.2	(4)	96.1 - 100
cis-platinum treated rabbits	96.7 ± 3.8	(4)	93.1 - 100
C. Gel chromatographic results (48 hours)			
control rabbits	96.1 ± 4.6	(4)	89.3 - 100
Cis-platinum treated rabbits	93.9 ± 10.2	(4)	78.8 - 100

The *in vitro* serum binding of ^{67}Ga-citrate was accomplished by sampling blood from the same rabbits before cis-platinum treatment and 24 hours after completion of treatment. These samples were incubated with ^{67}Ga-citrate for 3 hours before separation. As shown in Table 24, there is a definite increase in ^{67}Ga-citrate binding in post cis-platinum treatment serum in at least 3 of the rabbits. Using this paired *in vitro* comparison, it is apparent that ^{67}Ga-citrate more readily binds to serum from treated rabbits compared to pre-treatment

controls.

Table 24. Protein binding of ^{67}Ga-citrate after three hours of incubation with sera obtained from rabbits before and after cis-platinum treatment.

	Percent bound (mean ± s.d.) in individual rabbits designated by letters			
A. Dialysis results				
	A	B	C	D
before cis-platinum treatment	8.1 ±1.3	10.8 ±0.4	10.7 ±0.8	11.3 ±0.8
24 hours after cis-platinum treatment	31.3 ±4.3	24.3 ±2.0	11.4 ±0.8	53.9 ±8.5
B. Gel chromatographic results				
before cis-platinum treatment	85.0	80.9	84.3	92.0
24 hours after cis-platinum treatment	100	85.3	91.1	100

This effect may be understood by analysis of the data in Table 25, which shows the serum iron parameters from the control and cis-platinum treated rabbits. There is a significant reduction (~25%) in serum iron and percent saturation between the two groups. Thus a drop in serum iron levels is reflected by a reduced saturation of serum transferrin and a greater ability to bind available ^{67}Ga.

Table 25. Serum iron parameters from control and cis-platinum treated rabbits.

Parameter (units)	Control rabbits mean ± s.d.	(n)	Cis-platinum treated rabbits mean ± s.d.	(n)
serum iron** (μmole/dl)	36.3 ± 8.0	(14)	27.4 ± 4.3	(8)
TIBC (μmole/dl)	61.6 ± 8.5	(15)	62.6 ± 10.0	(8)
saturation* (percent)	56.0 ± 10.9	(14)	44.9 ± 9.5	(8)
hematocrit (percent)	45 ± 3	(4)	44 ± 3	(4)

** $p < 0.025$; * $p < 0.05$
TIBC = Total Iron Binding Capacity

The relationship between the percent binding of ^{67}Ga to serum proteins from the dialysis results and serum unsaturated iron binding capacity (UIBC) calculated from the iron parameters is shown in Figure 20. In both the *in vitro* and *in vivo* cases, there is a significant correlation between the two indices. This type of relationship has been implied in another study, strictly for *in vitro* binding of ^{67}Ga-citrate with varying degrees of iron labeled serum[629]. It was previously shown that serum cis-platinum has no effect on ^{67}Ga-citrate binding *in vitro*, thus the reduced iron levels induced by cis-platinum treatment are likely responsible for this effect. Patients with true positive ^{67}Ga-citrate scans have been shown to have reduced iron, transferrin and ferritin levels in their serum compared to appropriately matched controls[424]. The influence of the nature of the disease and/or the treatment may be responsible for these changes but

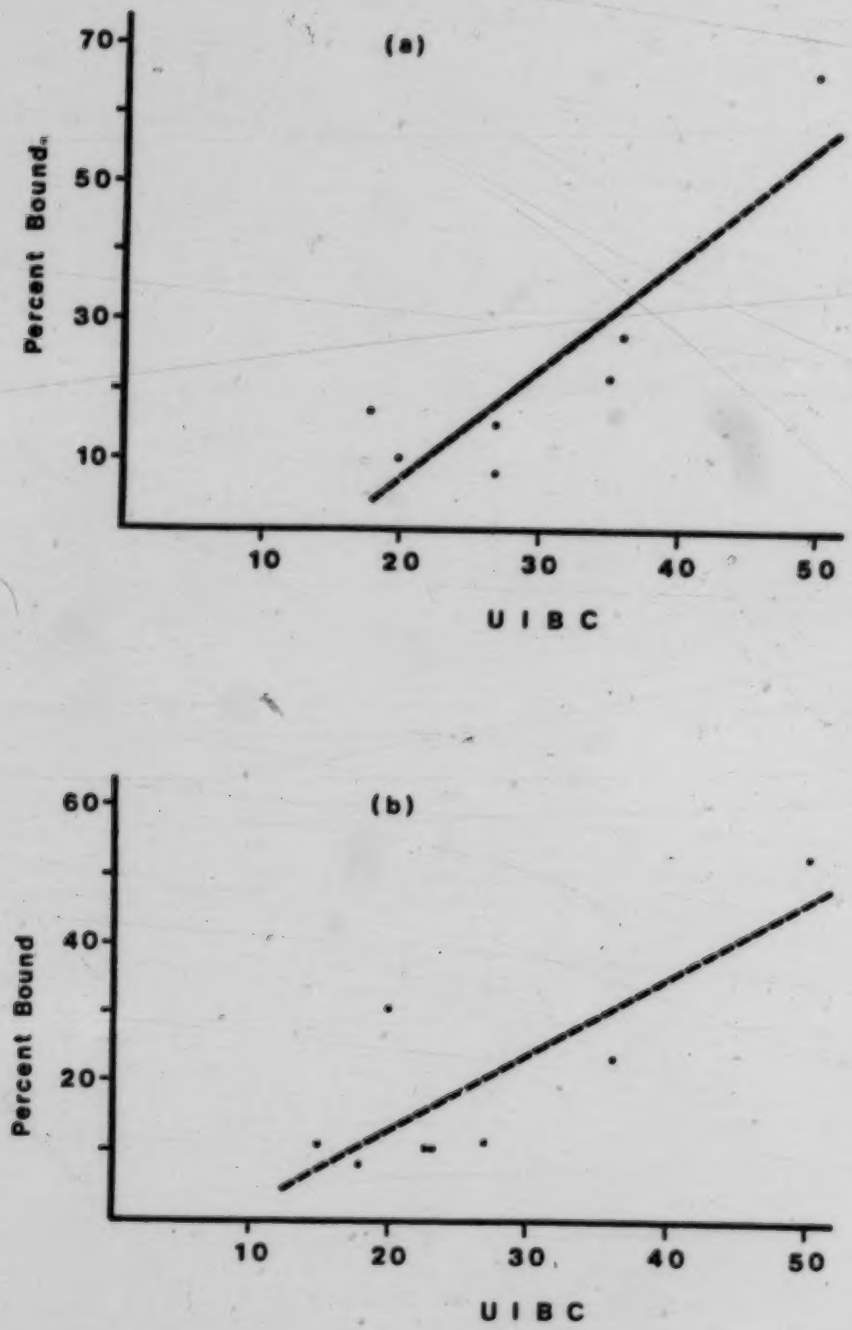


Figure 20. Protein binding of Ga-67 (a) in vivo and (b) in vitro versus the unsaturated iron binding capacity (UIBC) of the serum.

in the case of the cis-platinum treated rabbits, the effects of changes to similar iron parameters is a discernible increase in ^{67}Ga binding to serum components.

There may be several physiological mechanisms responsible for the reduction in rabbit serum iron levels and a concomittant drop in transferrin saturation. The depression of the hematopoietic system[259] and the renal impairment known to result from cis-platinum treatment are likely involved. The direct destruction of red blood cell precursors[297] and the suppressed release of erythropoietin due to damaged kidneys[638] would both lead to a decrease in serum iron levels. However, these animals were not frankly anemic since hematocrit values were not significantly different from controls (Table 25).

Another cause of lowered serum iron levels may lie in drug induced disruption of diet and absorption processes. Cis-platinum produces a characteristic anorexic effect in rabbits shown in Figure 21. This results in a short term deprivation of dietary iron which may be sufficient to reduce iron levels by the observed degree. Dietary restrictions have also been shown to reduce transferrin synthesis in the rat[639], however, since the total unsaturated iron binding capacity (TIBC) did not change with cis-platinum treatment, we may assume this process does not occur in rabbits under these conditions. Cis-platinum also causes sloughing of the columnar villi cells of the small intestine[258] which control the vascular incorporation of ingested iron[640]. Thus, impairment of this absorption mechanisms would also tend to lower serum iron levels. The administration of cis-platinum to rats has also been shown to decrease iron levels and was attributed to inflammation of the kidney,

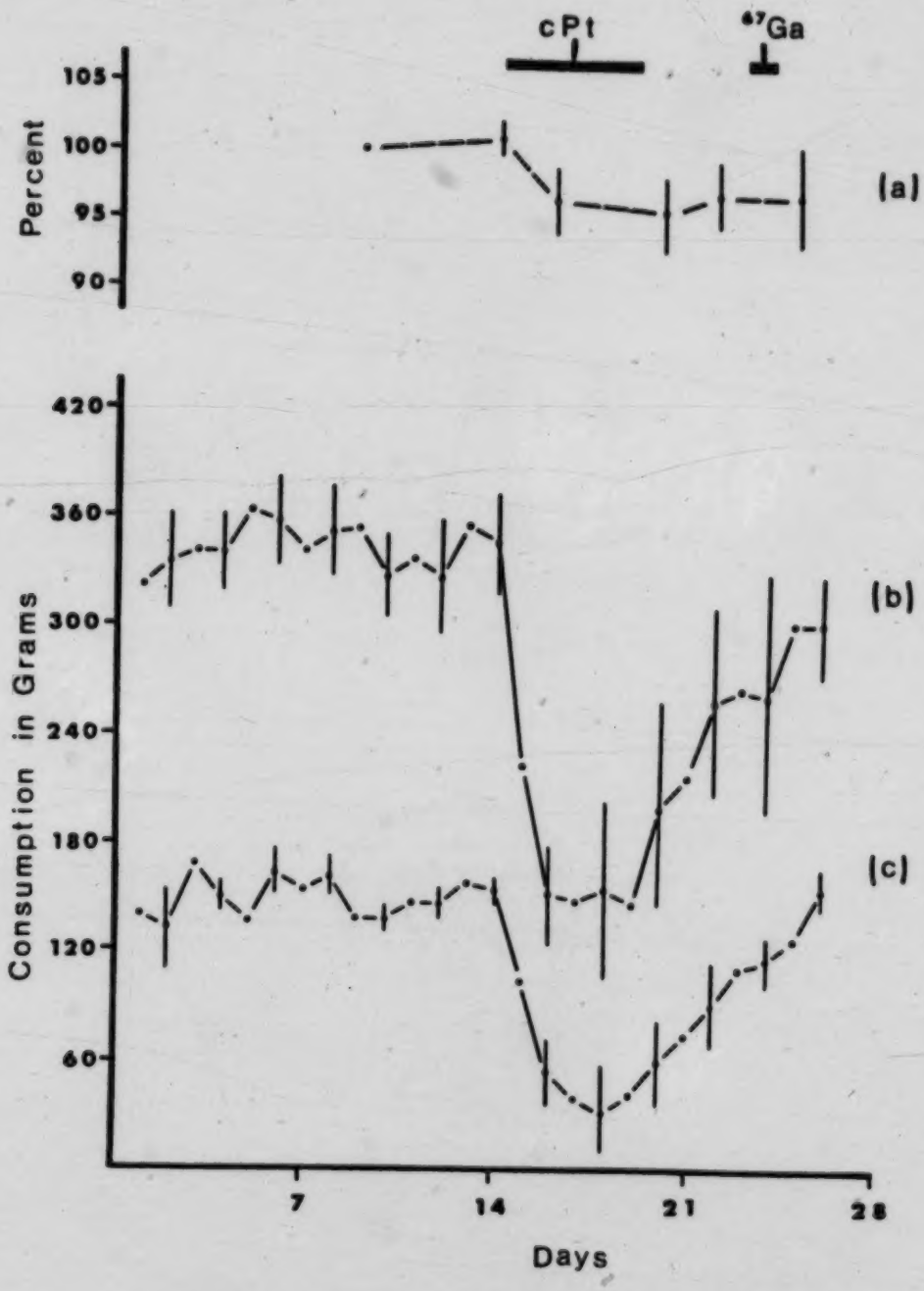


Figure 21. Percent changes in body weight (a); water (b) and food (c) consumption in rabbits during the cis-platinum treatment phase. (mean ± s.e.m. ; n=4-8)

gastrointestinal tract and liver, since inflammatory conditions are known to alter iron concentrations in this manner[641], and may be involved in this effect in rabbits.

3. Whole Body Elimination

Whole body elimination of ^{67}Ga was assessed by daily measurements of total body activity for 7 days after ^{67}Ga-citrate administration. The retention of ^{67}Ga was significantly greater in cis-platinum treated animals compared to the control phase, as shown in Figure 22. The corresponding elimination parameters derived from these curves are shown in Table 26, and indicate in the control phase about one-half of ^{67}Ga leaves the body with a half-life of about 21 hours, while the remainder is removed much more slowly with a half-life of about two weeks. However, in cis-platinum treated rabbits, all ^{67}Ga is eliminated at a single exponential rate with a half-life of about 12 days. This observed lack of a fast elimination phase results in an immediate increase in whole body retention apparent within 24 hours after ^{67}Ga-citrate administration.

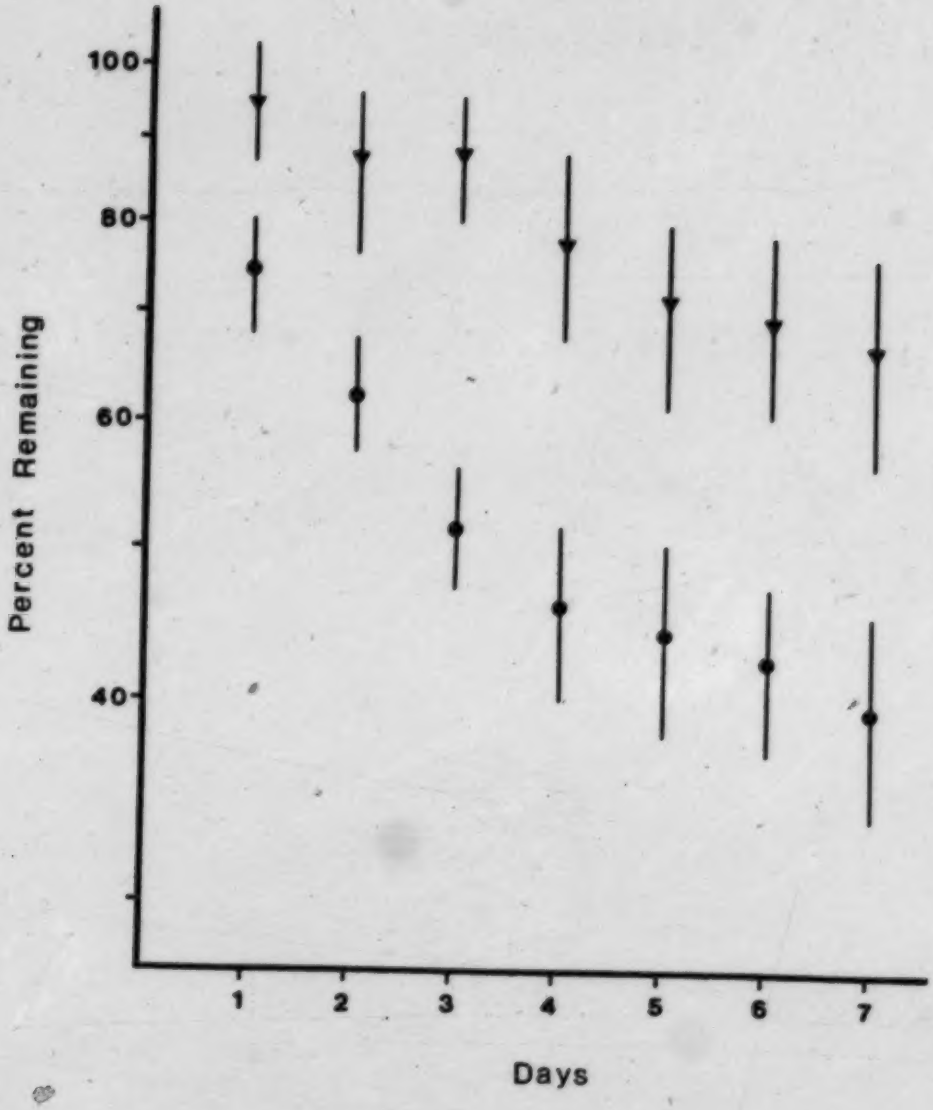


Figure 22. Whole body elimination of Ga-67 at various times after Ga-67 citrate administration in control (●) and cis-platinum treated (▼) rabbits. (mean ± s.d.; n=7-8)

Table 26. Whole body elimination parameters of ^{67}Ga from ^{67}Ga-citrate administration before and after cis-platinum treatment in rabbits.

Parameter (units)	Before cis-platinum treatment mean ± s.d.	(n)	After cis-platinum treatment mean ± s.d.	(n)
$t_{1/2}$ (hours)	20.8 ± 5.7	(7)	-	
%	44.7 ± 10.5	(7)	-	
$t_{1/2}$ (days)	13.5 ± 2.4	(7)	12.1 ± 5.4	(8)
%	55.3 ± 10.5	(7)	100 ± 4.7	(8)

4. Fecal and Urinary Excretion

A comparison of the effect of cis-platinum treatment on urinary and fecal excretion of ^{67}Ga is shown in Figure 23. Renal excretion of the tracer was significantly less than in the control phase at 24 and 48 hours, but thereafter the two groups maintained a similar pattern. Fecal excretion was also diminished for the first 24 hours, but by 4 days, excretion by this route is significantly greater than in controls. Thus, the observed increase in whole body retention after cis-platinum treatment is due primarily to a decrease in urinary excretion and a smaller drop in fecal elimination within the first two days after ^{67}Ga-citrate administration.

The causative factors involved in the elimination and excretion changes could be related to impaired renal function, enhanced protein binding and possible gastrointestinal developments. The effect of cis-platinum treatment on serum creatinine values is shown in Table 27. This parameter is a good measure of renal function, and the

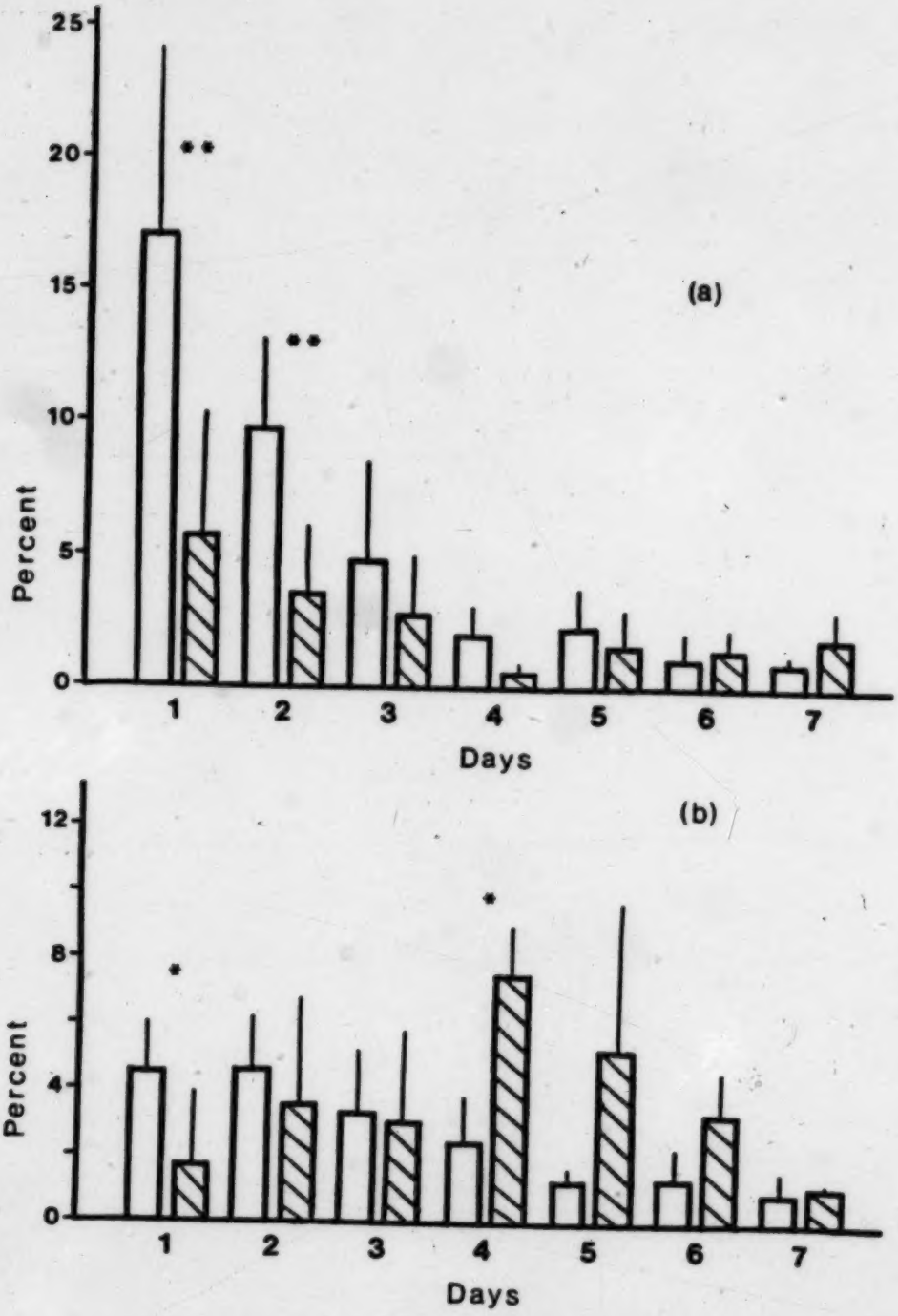


Figure 23. Urinary (a) and fecal (b) excretion of Ga-67 at various times after Ga-67 citrate administration in control (open bars) and cis-platinum treated (hatched bars) rabbits. (mean ± s.d. ; * $p < 0.05$; ** $p < 0.01$; n=2-5)

elevated value clearly indicates an altered ability of the kidney to clear this substance from the plasma compartment, and one would expect a similar detrimental effect on ^{67}Ga clearance as well. Further evidence of the renal damage was obtained from pathological analysis of kidney sections from cis-platinum treated rabbits. Several animals showed varying degrees of intestinal nephritis involving the cortex and particularly the distal medulla. In some animals, there was also evidence of tubular necrosis indicated by disruption of cells lining the proximal convoluted tubules and sloughing of cells into the lumina of the collecting tubules and portions of the loop of Henle.

Table 27. Serum creatinine values for control and cis-platinum treated rabbits.

Parameters (units)	Control rabbits mean ± s.d. (n)	Cis-platinum treated rabbits mean ± s.d. (n)
Serum creatinine* (mg/dl)	1.3 ± 0.2 (18)	2.1 ± 1.3 (8)

*$p < 0.01$

The previous evidence of increased serum protein binding of ^{67}Ga could also be implicated. Protein bound compounds are not filtered by the glomerular apparatus and thus not readily excreted via the urine, and some degree of reduced renal elimination may be attributed to this behavior. Studies in rats with iron deficiency showed a smaller decrease in ^{67}Ga urinary excretion even when serum iron levels dropped to about half normal and the animals became frankly anemic[642]. In this case, only a 25% drop in serum iron levels was observed but

urinary excretion was diminished by almost 70%, and the rabbits were not anemic.

Whole body retention of ^{67}Ga has been reported to be increased by animal fasting prior to ^{67}Ga-citrate administration[486]. Since cis-platinum treated rabbits consumed significantly less food (and water) than the control group, one may suspect a similar effect. However, it would seem this phenomenon is caused by the subsequent changes in dietary iron intake and in turn, reduced serum iron levels and enhanced ^{67}Ga protein binding as discussed earlier. Thus, dietary restrictions may indirectly affect urinary excretion and whole body retention.

The initial decline in fecal excretion is likely related to both the low gastrointestinal bulk caused by incomplete feeding and the intestinal damage associated with cis-platinum use. The rebound of fecal excretion 4 days after ^{67}Ga-citrate administration may be due to recovery of this system and elimination of ^{67}Ga through the most viable pathway or possibly via the bile from increased liver accumulation.

5. Urinary Disposition

The relative partitioning of ^{67}Ga between soluble and insoluble metabolites in rabbit urine was studied by a simple centrifugation technique. Figure 24 represents the solubility of endogenous ^{67}Ga activity in urine from both control and cis-platinum treated rabbits as a function of pH. Cis-platinum treatment did not significantly change the observed pH of 24 hour urine samples and thus, as expected, the degree soluble ^{67}Ga activity was also not altered. The ability of

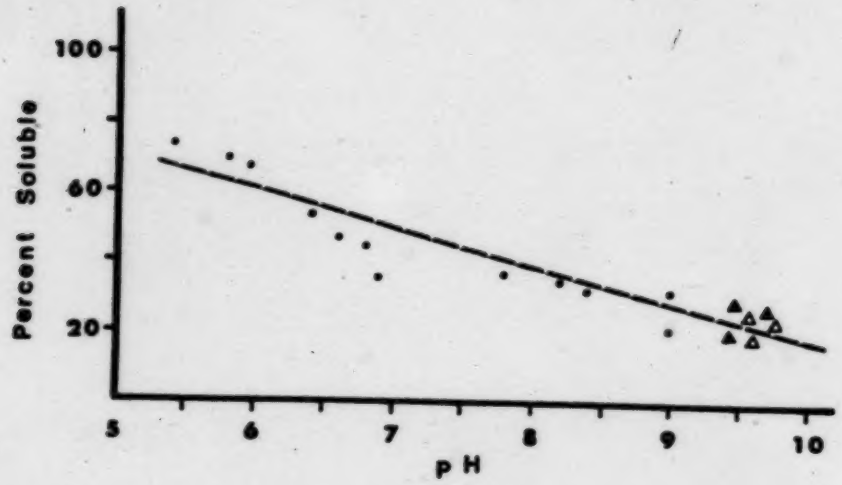


Figure 24. Percent solubility of endogenous Ga-67 in 24 hour urine samples versus externally adjusted pH (•). Percent solubility of endogenous Ga-67 and urinary pH in 24 hour samples from control (▲) and cis-platinum treated (△) rabbits.

cis-platinum to urinary pH has been documented in rats[303], however, in this case, a change in metabolism could only be detected by a change in urinary pH with a corresponding change in solubility which was significantly outside the area defined by the line in Figure 24.

The sediment portion of rabbit urine has been found to consist of crystals of ammonium magnesium phosphate (triple phosphate) and calcium carbonate (anhydrous and monohydrate)[643]. At the observed urinary pH values, ^{67}Ga may exist as a salt of either of these anions[243] or as gallate[371]. Stable gallium salts have been shown to be excreted as phosphates in the urine of rats[398], suggesting this form may be preferred. The renal elimination of ^{67}Ga is greatly reduced by cis-platinum treatment, however, it appears to affect the quantity rather than the quality of ^{67}Ga in the urine, since metabolites appear to be the same in both treated and control animals.

B. Pharmacokinetics

The computer analysis of ^{67}Ga blood levels versus time after ^{67}Ga-citrate administration revealed that ^{67}Ga pharmacokinetics in the rabbit were best described by a three exponential equation of the form:

$$C_B = A_1 e^{-\lambda_1 t} + A_2 e^{-\lambda_2 t} + A_3 e^{-\lambda_3 t}$$

where C_B is the percentage of the ^{67}Ga dose per gram of blood at time t; A_1, A_2 and A_3 are the pre-exponential coefficients and λ_1, λ_2 and λ_3 are the exponential rate constants.

Figure 25 shows the computer fitted curves along with the actual data points for a typical rabbit. Correlation coefficients were very high ($r > 0.98$) indicating good agreement between the measured data and the computer fitted curves and parameter estimates. The data from each rabbit were analyzed individually to give the most realistic measure of statistical uncertainty. The cumulative curve, generated from the averages of each parameter from all rabbits is shown in Figure 26. Both before and after cis-platinum treatment, ^{67}Ga blood levels showed a triphasic decline, with the only significant difference between the groups occurring sometime after 48 hours. In cis-platinum treated rabbits, ^{67}Ga clearance becomes slower during the terminal phase as shown in Table 28 by the significantly smaller rate constant (λ_3) for this phase. Using additional data of ^{67}Ga blood levels from sacrificed rabbits, the blood concentration at 48 hours are significantly greater than those from the control rabbits at this time (Table 29). Thus, although cis-platinum treatment produces no immediate detectable impact on ^{67}Ga blood levels, an increase in vascular ^{67}Ga can be demonstrated a few days after ^{67}Ga-citrate administration when the overall blood clearance becomes slower. Patients undergoing cis-platinum therapy have been observed to have an increased blood background of ^{67}Ga during scanning procedures[2].

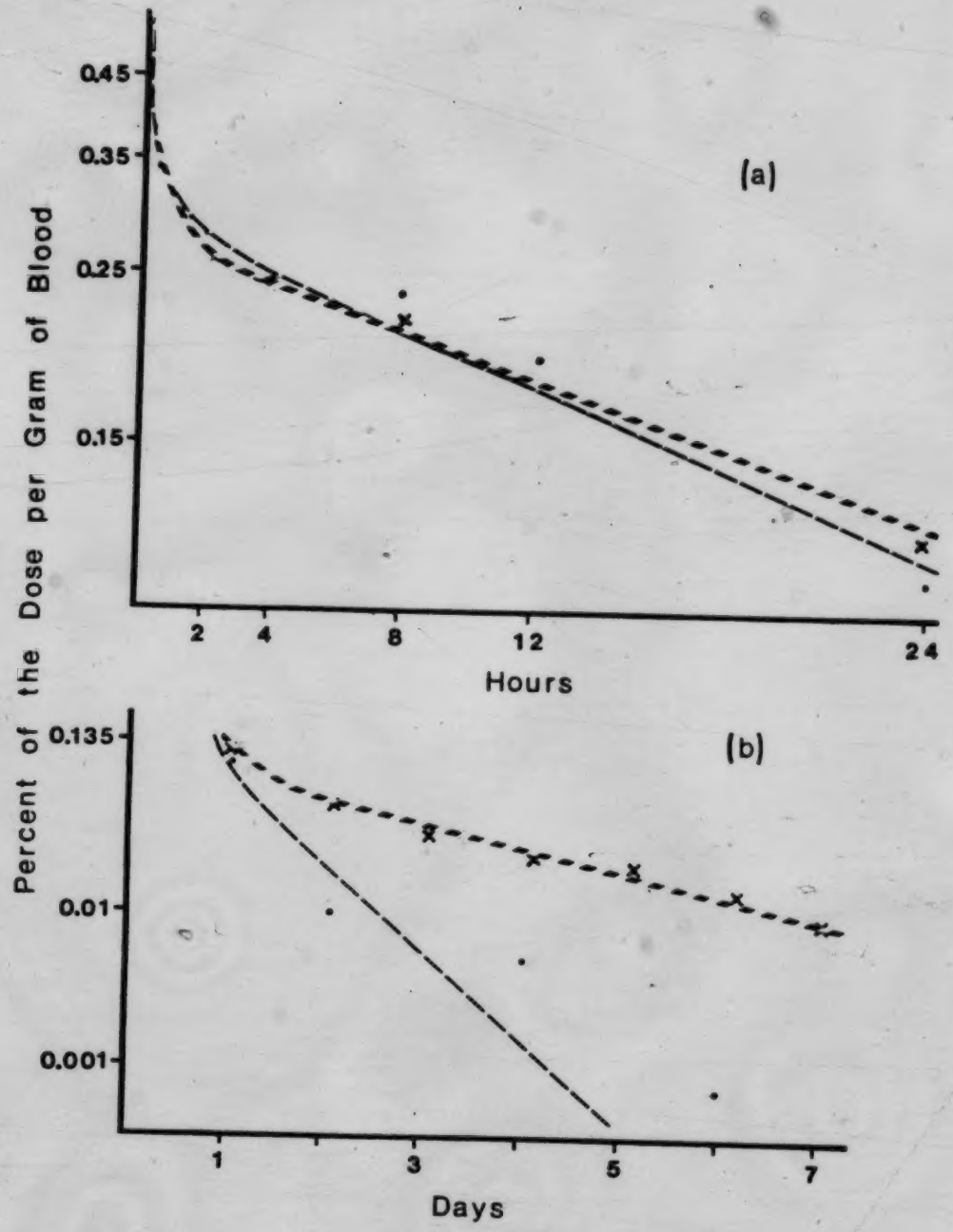


Figure 25. Measured blood levels of Ga-67 and the corresponding computer fitted curves for the time periods (a) 0-24 hours and (b) 1-7 days after Ga-67 citrate administration ; before (•, ——) and after (×, - - -) cis-platinum treatment in a typical rabbit.

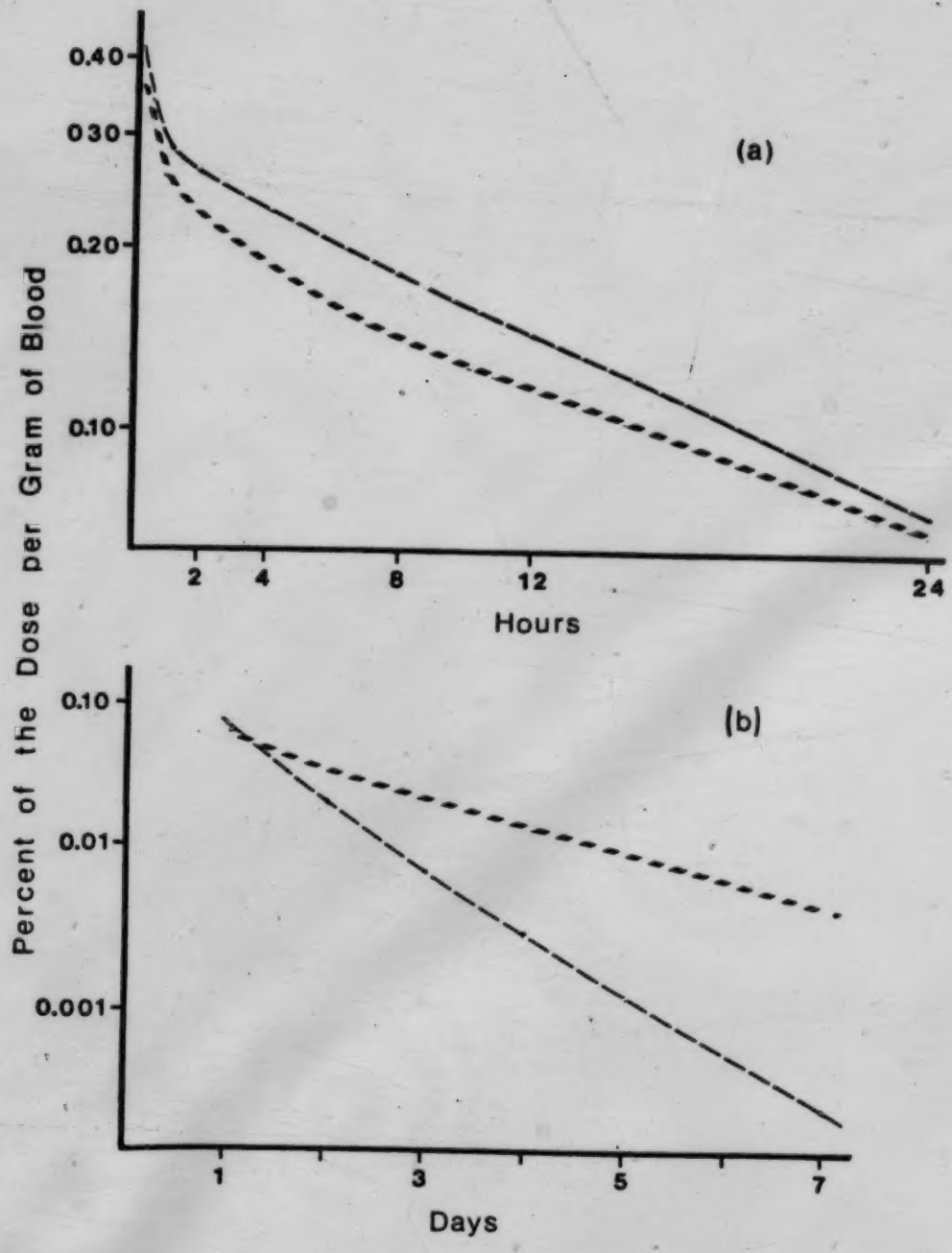


Figure 26. Computer curves of Ga-67 blood levels versus time after Ga-67 citrate administration generated from the cumulative pharmacokinetic parameters obtained before (————) and after (– – –) cis-platinum treatment in rabbits.

Table 28. Effect of cis-platinum treatment on selected pharmacokinetic parameters of ^{67}Ga-citrate in rabbits.

Parameter (units)	Control rabbits mean ± s.d.	(n)	Cis-platinum treated rabbits mean ± s.d.	(n)
λ_3 (hours^{-1})	3.61 ± 1.65	(4)	2.06 ± 0.60	(4)
λ_2 (hours^{-1})	0.073 ± 0.023	(4)	0.123 ± 0.079	(4)
λ_1* (hours^{-1})	0.036 ± 0.006	(4)	0.018 ± 0.008	(4)

* $p < 0.05$ paired t test

These pharmacokinetic results can likely be traced to the enhanced protein binding and reduced elimination of ^{67}Ga previously demonstrated in cis-platinum treated rabbits. The singular effect of markedly raised serum UIBC values in rabbits has been shown to increase blood concentrations of 67Ga[517] which supports the contribution of a more highly serum bound ^{67}Ga to this effect. Thus, both of these factors favour elevated blood levels of the tracer, but at early time periods, this may be balanced by a more rapid tissue uptake because of its greater protein bound fraction, or its slightly longer residence time in the circulating pool. By 2-3 days, the uptake processes are complete and perhaps into a washout phase[373], at which time blood clearance becomes dominant and the cis-platinum induced impairment of these processes causes blood clearance to become slower compared to controls.

Table 29. Blood levels of ^{67}Ga in control and cis-platinum treated rabbits.

Sampling time (hrs)	Control rabbits mean ± s.d.	(n)	Cis-platinum treated rabbits mean ± s.d.	(n)
4	0.223 ± 0.018	(4)	0.195 ± 0.040	(4)
8	0.207 ± 0.021	(4)	0.160 ± 0.042	(2)
24	0.086 ± 0.018	(4)	0.081 ± 0.028	(4)
48*	0.029 ± 0.020	(8)	0.082 ± 0.051	(7)

* $p < 0.05$

7. Whole Body Distribution

The whole body distribution of ^{67}Ga 48 hours after ^{67}Ga-citrate injection is shown in Table 30. In almost all cases, there is a significant increase in the tissue uptake of ^{67}Ga after cis-platinum treatment. This rather generalized increase in ^{67}Ga uptake (with exceptions) likely represents extra accumulation of ^{67}Ga which is normally removed from the system by the kidney or intestinal routes, since overall body burdens of the tracer in these rabbits was significantly elevated relative to controls. In the absence of adequately functioning elimination processes, tissue uptake is readily capable of competing for plasma ^{67}Ga. The relative uptake in each tissue of the cis-platinum treated rabbits compared to the corresponding control tissues is shown in Table 31. Treatment factors generally range from 2-3 indicating a double to triple increase in ^{67}Ga accumulation. Comparative whole body elimination in each group of rabbits differed by a similar amount, lending support to this

mechanism as the primary cause for the observed general increase in tissue uptake. This reasoning was also suggested in the previous mouse study where a nonspecific increase after cis-platinum treatment was also observed.

Since blood levels are also increased in the tissues of cis-platinum treated rabbits, one may expect organ blood content to contribute to the overall increase. This is likely the case for tissues with very high blood content such as lungs and which show a correspondingly high treatment factor. However, even for liver which has a relatively high plasma/tissue ratio (~0.18)[517], this represents only a 14% reduction of the ^{67}Ga tissue uptake value, and thus this effect likely contributes much less than 10% to most other tissues.

The second factor which contributes to the generalized increase in tissue uptake after cis-platinum treatment is the slightly greater extent of plasma protein bound ^{67}Ga. It has been shown that rabbits with raised serum UIBC values have greater soft tissue uptakes compared to animals with lower values, although only slight changes in serum protein binding could be demonstrated[517].

The cis-platinum induced dietary restrictions may also indirectly influence whole body distributions since fasted mice have been shown relatively high levels of ^{67}Ga in liver, kidney, spleen and blood[486]. As discussed previously, low food ingestion may subsequently result in reduced serum iron levels, greater ^{67}Ga protein binding and concommittant changes in tissue uptake. At the time of ^{67}Ga-citrate injection, food (and water) intake had resumed near normal amounts such that immediate effects should not be a factor. The nutritional lack as a result of reduced food intake causes only slight changes in

whole body weight (Figure 21) and this does not play a major role in the generalized increase in ^{67}Ga tissue uptake. Whole body scans of a typical rabbit taken at 12, 24 and 48 hours after ^{67}Ga-citrate administration before and after cis-platinum treatment are shown in Plate 1. Although difficult to interpret visually, disc stored data indicates greater tissue uptake occurs at about 24-48 hours, in confirmation of the animal sacrifice data.

A few tissues show more pronounced increases in ^{67}Ga tissue uptake due to cis-platinum treatment relative to the others and may represent more selective effects of the drug. The known influence of cis-platinum on the intestinal tract[258] and renal tubules (shown previously) may explain the high stomach, intestine and kidney medulla treatment factors. A combination of increased blood flow and thus improved tracer delivery and the known accumulation of ^{67}Ga at inflammatory foci are likely involved.

Only two tissues showed no difference in ^{67}Ga tissue uptake after cis-platinum treatment. Whole bone (ribs) uptake may be influenced by several biological parameters. Iron deficient rats with elevated UIBC values showed no changes in femoral accumulation of ^{67}Ga[642], but studies of rabbits with raised UIBC values indicated decreased bone uptake but increased bone marrow levels[517]. Thus, the whole bone sampling done here and in the rat study may simply represent a balance between marrow and structural bone uptake. Alternately, the expected decrease in bone uptake due to enhanced ^{67}Ga protein binding may be offset by the predicted increased uptake due to elevated whole body levels. Ribs may not be representative of peripheral skeletal uptake since whole body image data from one rabbit showed increased uptake of

^{67}Ga in the femur after cis-platinum treatment. The mechanism responsible for the lack of expected higher uptake in the uterus of treated rabbits can only be speculated upon as relating to the known affinity of the drug for this tissue[27] and a suspected cellular damage which inhibits accumulation processes.

Table 30. Tissue distribution of ^{67}Ga in control and cis-platinum treated rabbits 48 hours after ^{67}Ga-citrate administration.

Tissue	% dose per gram of tissue x 10^{-2} Control (mean ± s.d; n=4)	After Cis-platinum treatment* (mean ± s.d; n=4)
heart	2.19 ± 1.35	5.36 ± 1.43
lung	2.33 ± 1.18	7.42 ± 2.02
liver	5.06 ± 1.77	15.14 ± 3.12
blood	3.65 ± 2.34	11.60 ± 3.89
stomach	5.10 ± 1.16	19.22 ± 4.84
spleen	5.91 ± 2.43	14.60 ± 4.17
pancreas	1.04 ± 0.28	2.28 ± 0.44
muscle	0.13 ± 0.04	0.34 ± 0.16
L. intestine (descending colon)	0.31 ± 0.09	1.09 ± 0.41
S. intestine (duodenum)	1.16 ± 0.33	1.83 ± 0.39
bone (ribs)	12.10 ± 2.75	12.86 ± 3.24
ovaries	4.03 ± 1.74	12.07 ± 3.20
uterus	2.74 ± 1.71	3.87 ± 1.47
kidney		
- medulla	3.98 ± 1.15	14.78 ± 5.50
- cortex	15.45 ± 5.18	30.83 ± 3.12
- whole	13.96 ± 3.88	25.99 ± 2.46

*All tissues are significantly different from controls ($p < 0.025$) except muscle and small intestine ($p < 0.05$). Uterus and bone are not significantly different from controls.

Table 31. Relative tissue uptake of ^{67}Ga in control and cis-platinum treated rabbits 48 hours after ^{67}Ga-citrate administration.

Tissue	Treatment* Factor	Tissue	Treatment* Factor
heart	2.6	L. intestine (descending colon)	3.4
lung	3.2	S. intestine (duodenum)	1.6
liver	3.0	bone (ribs)	-
blood	3.2	ovaries	3.0
stomach	3.8	uterus	-
spleen	2.4	kidney	
pancreas	2.2	- medulla	3.7
muscle	2.6	- cortex	2.0
		- whole	1.9

$$\text{* Treatment Factor} = \frac{\text{mean of \% dose per gram of tissue of treated animals}}{\text{mean of \% dose per gram of tissue of control animals}}$$

where difference between control and treated tissues was significant.

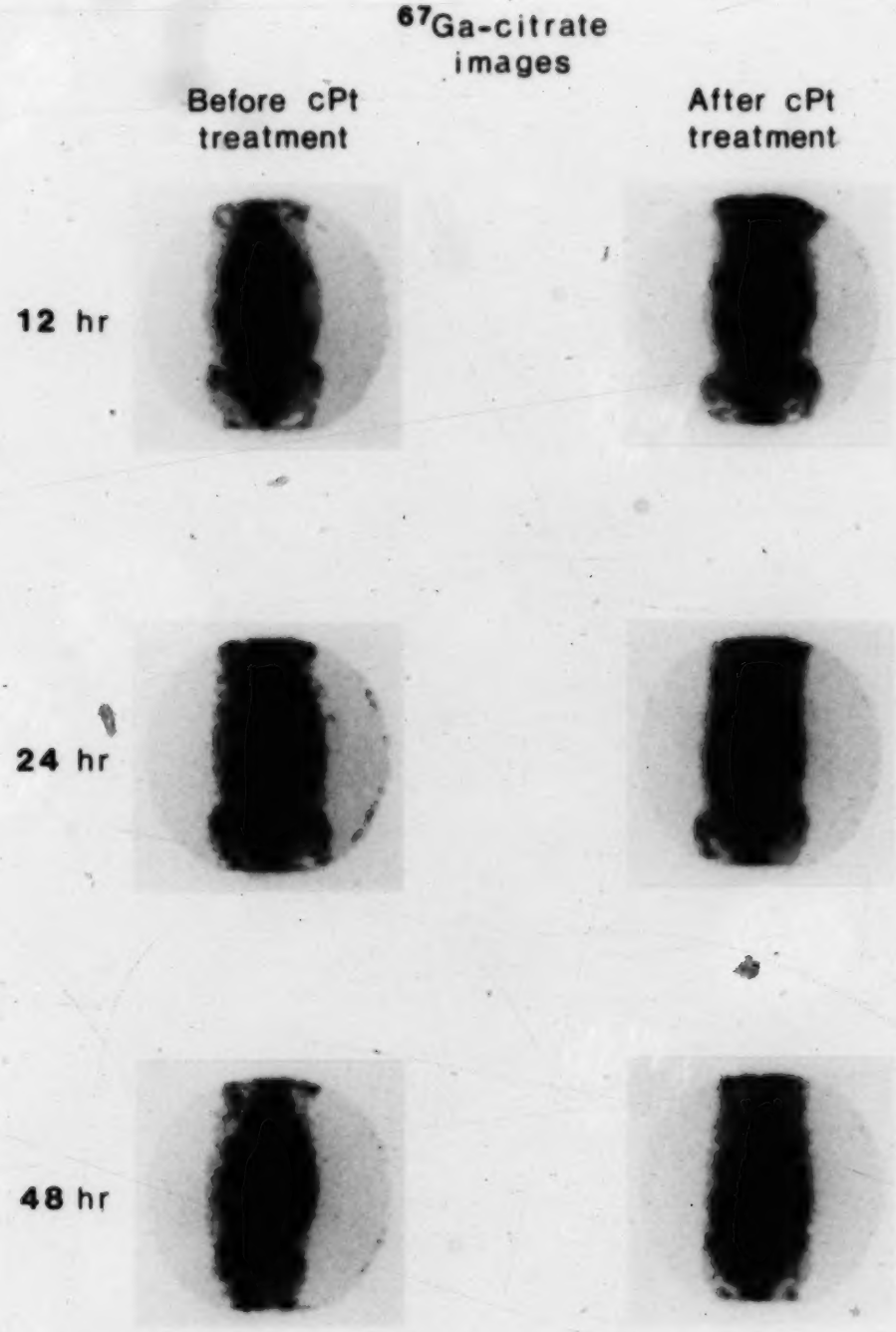


Plate 1. Gamma camera images of Ga-67 at various times after Ga-67 citrate administration to a typical rabbit before and after cis-platinum (cPt) treatment.

SUMMARY AND CONCLUSIONS

This thesis was concerned with two major topics. The first was devoted to the development and comparison of radioanalytical techniques for the determination of the chemotherapeutic drug cis-platinum in biological samples. A post-thermal neutron irradiation solvent extraction technique was derived which effectively enables low level platinum analysis by removing all major radionuclidic interferences present in the matrix. Labeled cis-platinum was prepared by several routes, the preferred method under our conditions being the direct thermal neutron irradiation of the purified compound and solid scintillation detection of the X-ray and low energy gamma emissions.

The second major topic involved the influence of this drug on the biological behavior of the radiopharmaceutical ^{67}Ga-citrate. The possible effects of cis-platinum and a related platinum compound, K_2PtCl_4 on ^{67}Ga plasma transport was studied in vitro under specific conditions using buffered transferrin solutions and human serum with two methods of free and bound component separation. It was observed that although some degree of inhibition of ^{67}Ga binding to transferrin by platinum compounds took place in isolated transferrin solutions, this process did not occur in whole serum in vitro. In vivo studies in rats and mice revealed the most dramatic effects were demonstrated when the drug was given some time before ^{67}Ga-citrate such that the development of drug induced toxicities were likely implicated. In the rabbit model, these toxicities were found to involve changes in renal function, in serum iron parameters and in dietary consumption which influenced the biological handling of a subsequently administered dose of ^{67}Ga-citrate. This effect was demonstrated as reduced excretion, slightly enhanced protein binding, elevated tissue levels and

altered blood kinetics of the tracer. Thus, the influence of cis-platinum treatment on the biological behavior of ^{67}Ga-citrate in animals was shown to be predominantly the indirect result of delayed toxicities of the drug. This information will likely be of value in the correct interpretation of human ^{67}Ga-citrate scans of patients previously or concurrently receiving cis-platinum.

BIBLIOGRAPHY

1. Hayes R.L., Edwards C.L., New applications of tumour-localizing radiopharmaceuticals, in "Medical Radioisotope Scintigraphy 1972", Vol. II, IAEA, 1973, pp. 531-552.

2. Lentle B.C., Scott J.R., Noujaim A.A., et. al., Iatrogenic alterations in radionuclide biodistributions. Sem. Nucl. Med. 9:131 1979.

3. Hladik W.B., Nigg K.K., Rhodes B.A., Drug induced changes in the biologic distribution of radiopharmaceuticals. Sem. Nucl. Med. 12:184 1982.

4. Rosenberg B., Van Camp L., Krigas T., Inhibition of cell division in Escherichia coli by electrolysis products from a platinum electrode. Nature 205:698 1965.

5. Rosenberg B., Van Camp L., Grimbley E.B., et. al., The inhibition of growth or cell division in Escherichia coli by different ionic species of platinum (IV) complexes. J. Biol. Chem. 242:1347 1967.

6. Rosenberg B., Renshaw E., Van Camp L., et. al., Platinum induced filamentous growth in Escherichia coli. J. Bacteriol. 93:716 1967.

7. Howle J.A., Gale G.R., cis-Dichlorodiammineplatinum (II): cytological changes induced in Escherichia coli. J. Bacteriol. 103:258 1970.

8. Moore R.L., Brubaker R.R., Effect of cis-platinum (II) diamminodichloride on cell division of Hyphomicrobium and Caulobacter. J. Bacteriol. 125:317 1976.

9. Rosenberg B., Van Camp L., Trosko J.E., Platinum compounds: a new class of potent antitumor agents. Nature 222:385 1969.

10. Von Hoff D.D., Rozencweig M., cis-Diamminedichloroplatinum (II): A metal complex with significant anticancer activity. Adv. Pharmacol. Chemother. 16:273 1979.

11. Truter M.R., Milburn C.W., The crystal structure of cis and trans-dichloro diammine platinum (II). J. Chem. Soc. A:1609 1966.

12. LeRoy A.F., Some quantitative data on cis-dichlorodiammineplatinum (II) species in solution. Cancer Treat. Rep. 63:231 1979.

13. Boreham C.J., Broomhead J.A., Fairlie D.P., A ^{195}Pt and ^{15}N N.M.R. study of the anticancer drug, cis-diamminedichloroplatinum (II), and its hydrolysis and oligomerization products. Aust. J. Chem. 34:659 1981.

14. Hincal A.A., Long D.F., Repta A.J., Cisplatin stability in aqueous parenteral vehicles. J. Parenteral Drug Assoc. 33:107 1979.

15. Johnson N.P., Hoeschele J.D., Rahn R.O., Kinetic analysis of the in vitro binding of radioactive cis- and trans- dichlorodiammineplatinum (II) to DNA. Chem. Biol. Interact. 30:151 1980.

16. Rosenberg B., Anticancer activity of cis-dichlorodiammineplatinum (II) and some relevant chemistry. Cancer Treat. Rep. 63:1433 1979.

17. Rosenberg B., Noble metal complexes in cancer chemotherapy. Adv. Exp. Med. Biol. 91:129 1977.

18. Inagaki K., Kidani Y., Suzuki K., et. al., Platinum complexes of diamminocarboxylates and their ethyl ester derivatives: antitumor activity and interaction with deoxyribonucleic acid. Chem. Pharm. Bull. 28:2286 1980.

19. Thompson A.J., Williams R.J., Resolva S., The chemistry of complexes related to cis- $Pt(NH_3)_2Cl_2$. An antitumor drug. Struct. Bonding 1:1 1972.

20. Cleare M.J., Hoeschele J.D., Relationship between structure and activity. Platinum Met. Rev. 17:2 1973.

21. Cleare M.J., Hydes P.C., Malerbi B.W., et. al., Antitumor platinum complexes: relationships between chemical properties and activity. Biochimie 60:835 1978.

22. Leh K., Wolf W., Platinum complexes: a new class of a antineoplastic agents. J. Pharm. Sci. 65:315 1976.

23. Basolo F., Pearson R.G., "Mechanisms of Inorganic Reactions" 2nd ed. Wiley, New York, N.Y., 1967, p. 23-355.

24. Basolo F., Gray H.B., Pearson R.G., Mechanism of substitution reactions of complex ions. XVII. Rates of reaction of some platinum (II) and palladium (II) complexes with pyridine. J. Am. Chem. Soc. 82:4200 1960.

25. Cleare M.J., Hoeschele J.D., Studies on the antitumor activity of group VIII transition metal complexes. Part 1. Platinum (II) complexes. Bioinorg. Chem. 2:187 1973.

26. Rosenberg B., Platinum coordination complexes in cancer chemotherapy. Naturwissenschaften 60:399 1973.

27. Taylor D.M., The pharmacokinetics of cis-diammino-dichloroplatinum (II) in animals and man: relation to treatment scheduling. Biochimie 60:949 1978.

28. Lange R.C., Spencer R.P., Harder H.C., Synthesis and distribution of a radiolabeled antitumor agent: cis-diamminedichloroplatinum (II). J. Nucl. Med. 13:328 1972.

29. Hoeschele J.D., Van Camp L., Whole body counting and the distribution of cis-$^{195m}[Pt(NH_3)_2Cl_2]$ in the major organs of swiss white mice, in "Advances in Antimicrobial and Antineoplastic Chemotherapy", Vol. 2, M. Semonsky, M. Hejzlar, S. Masak, eds., University Park Press, Baltimore, 1972, pp. 241-2.

30. Hoeschele J.D., Butler T.A., Roberts J.A., Microscale synthesis and biodistribution of Pt-195m-labeled cis-dichlorodiammineplatinum (II), cis-DDP., in "Radiopharmaceuticals II: Proceedings of the Second International Symposium on Radiopharmaceuticals", V.J. Sodd, D.R. Allen, D.R. Hoogland, R.D. Ice, eds., Society of Nuclear Medicine, New York, N.Y., 1979, pp. 173-182.

31. Jones J.D., Robins A.B., Taylor D.M., Metabolism and cytotoxic effects of platinum antitumor agents in rats, in "Advances in Antimicrobial and Antineoplastic Chemotherapy", Vol. 2, M. Semonsky, M. Hejzlar, S. Masak, eds., University Park Press, Baltimore, 1972, pp. 229-30.

32. Taylor D.M., Jones J.D., Robins A.B., Metabolism of platinum [^{14}C] ethylenediammine dichloride in the rat. Biochem. Pharmacol. 22:833 1973.

33. Wolf W., Manaka R.C., Ingalls R.B., Radiopharmaceuticals in Clinical Pharmacology: ^{195m}Pt-cis-diamminedichloroplatinum (II), in "Radiopharmaceuticals and Labelled Compounds" Vol. II, IAEA, Vienna, 1973, pp. 205-221.

34. Wolf W., Manaka R.C., Synthesis and distribution of ^{195m}Pt cis-dichlorodiammine platinum (II). J. Clin. Hematol. Oncol. 7:79 1977.

35. Litterest C.L., LeRoy A.F., Guarino A.M., Disposition and distribution of platinum following parenteral administration of cis-dichlorodiammeplatinum to animals. Cancer Treat. Rep. 63:1485 1979.

36. Litterest C., Torres I.J., Guarino A.M., Plasma levels and organ distribution of platinum in the rat, dog, and dogfish shark following single intravenous administration of cis-dichlorodiammineplatinum II. J. Clin. Hematol. Oncol. 7:169 1977.

37. Litterest C.J., Gram T.E., Dedrick R.L., et. al., Distribution and disposition of platinum following intravenous administration of cis-diamminedichloroplatinum (II) (NSC-119875) to dogs. Cancer Res. 36:2340 1976.

38. LeRoy A.F., Lutz R.J., Dedrick R.L., et. al., Pharmacokinetic study of cis-dichlorodiammineplatinum (II) (DDP) in the beagle dog: thermodynamic and kinetic behavior of DDP in a biologic millieu. Cancer Treat. Rep. 63:59 1979.

39. Cvitkovic E., Spalding J., Bethune C.P., et. al., Improvement of cis-dichlorodiammineplatinum (NSC-119875): therapeutic index in an animal model. Cancer 39:1357 1977.

40. Lange R.C., Spencer R.P., Harder H.C., The antitumor agent cis-$Pt(NH_3)_2Cl_2$: distribution studies and dose calculations for ^{193m}Pt and ^{195m}Pt. J. Nucl. Med. 14:191 1973.

41. Vermorken J.B., Van der Vijgh W.J., Pinedo H.M., Pharmacokinetic evidence for an enterohepatic circulation in a patient treated with cis-dichlorodiammineplatinum (II). Res. Comm. Chem. Pathol. Pharmacol. 28:319 1980.

42. Crom W.R., Evans W.E., Pratt C.B., et. al., Cisplatin disposition in children and adolescents with cancer. Cancer Chemother. Pharmacol. 6:95 1981.

43. Pratt C.B., Hayes A., Green A.A., et. al., Pharmacokinetic evaluation of cisplatin in children with malignant solid tumors: a phase II study. Cancer Treat. Rep. 65:1021 1981.

44. Gormley P.E., Gangji D., Wood J.H., et. al., Pharmacokinetic study of cerebrospinal fluid penetration of cis-diamminedichloroplatinum (II). Cancer Chemother. Pharmacol. 5:257 1981.

45. Stewart D.J., Leavens M., Maor M., et. al., Human central nervous system distribution of cis-diamminedichloroplatinum and use as a radiosensitizer in malignant brain tumors. Cancer Res. 42:2474 1982.

46. Bonnem E.M., Litterest C.L., Smith F.P., Platinum concentrations in human glioblastoma multiforme following the use of cisplatin. Cancer Treat. Rep. 66:1661 1982.

47. DeSimone P.A., Yancey R.S., Coupal J.J., et. al., Effect of a forced diuresis on the distribution and excretion (via urine and bile) of ^{195m}platinum cis-dichlorodiammineplatinum (II). Cancer Treat. Rep. 63:951 1979.

48. Lagasse L.D., Pretorius R.G., Petrilli E.S., et. al., The metabolism of cis-dichlorodiammineplatinum (II): distribution, clearance and toxicity. Am. J. Obstet. Gynecol. 139:791 1981.

49. Smith P.H., Taylor D.M., Distribution and retention of the antitumor agent ^{195m}Pt-cis-dichlorodiammineplatinum (II) in man. J. Nucl. Med. 15:349 1974.

50. Deconti R.C., Toftness B.R., Lange R.C., et. al., Clinical and pharmacological studies with cis-diamminedichloroplatinum (II). Cancer Res. 33:1310 1973.

51. Gormley P.E., Bull J.M., LeRoy A.F., et. al., Kinetics of cis-dichlorodiammineplatinum. Clin. Pharmacol. Therap. 25:351 1979.

52. Cano J.P., Catalin J., Rigault J.P., et. al., Pharmacokinetic study of cis-dichlorodiammineplatinum (II) in children after rapid infusion. Biomedicine 34:146 1981.

53. Gullo J.J., Litterest C.L., Maguire P.J., et. al., Pharmacokinetics and protein binding of cis-dichlorodiammineplatinum (II) administered as a one hour and as a twenty hour infusion. Cancer Chemother. Pharmacol. 5:21 1980.

54. Loo T.L., Hall S.W., Salem P., et. al., Clinical pharmacological and toxicological studies of cis-diamminedichloroplatinum (II) by continuous intravenous infusion. Biochimie 60:957 1978.

55. Casper E.S., Kelson D.P., Alcock N.W., et. al., Platinum concentrations in bile and plasma following rapid and 6-hour infusions of cis-dichlorodiammineplatinum (II). Cancer Treat. Rep. 63:2023 1979.

56. Patton T.F., Himmelstein K.J., Belt R., et. al., Plasma levels and urinary excretion of filterable platinum species following bolus injection and IV infusion of cis-dichlorodiammineplatinum (II) in man. Cancer Treat. Rep. 62:1359 1978.

57. LeRoy A.F., Wehling M., Gormley P., et. al., Quantitative changes in cis-dichlorodiammineplatinum (II) speciation in excreted urine with time after IV infusion in man: methods of analysis, preliminary studies, and clinical results. Cancer Treat. Rep. 64:123 1980.

58. Bannister S.J., Sternson L.A., Repta A.J., Measurement of free-circulating cis-dichlorodiammineplatinum (II) in plasma. Clin. Chem. 23:2258 1977.

59. Long D.F., Repta A.J., Cis-platin: chemistry, distribution and biotransformation. Biopharm. Drug Dispos. 2:1 1981.

60. Cole W.C., Wolf W., Renal toxicity studies of protein-bound platinum (cis). Chem. Biol. Interact. 35:341 1981.

61. Manaka R.C., Wolf W., Distribution of cis-platin in blood. Chem. Biol. Interact. 22:353 1978.

62. Holdener E.E., Park C.H., Belt R.J., et. al., Effect of mannitol and human plasma on cytotoxicity of cis-dichlorodiammine-platinum. Clin. Res. 26:436A 1978.

63. Long D.F., Patton T.F., Repta A.J., Platinum levels in human erythrocytes following intravenous administration of cisplatin: importance of erythrocytes as a distribution site for platinum species. Biopharm. Drug Dispos. 2:137 1981.

64. Horer O.L., Nicolau C., Spectroscopic evidence for cis-[Pt $Cl_2(NH_3)_2$] erythrocyte membrane interaction. FEBS Lett. 14:262 1971.

65. Himmelstein K.J., Patton T.F., Belt R.J., et. al., Clinical kinetics of intact cisplatin and some related species. Clin. Pharmacol. Therap. 29:658 1981.

66. Belt R., Himmelstein K.J., Patton T.F., et. al., Pharmacokinetics of nonprotein bound species following administration of cis-dichlorodiammineplatinum (II). Cancer Treat. Rep. 63:1515 1979.

67. Sodhi A., Prasad S.B., Ultrastructural and fluorescence microscopial observations on the effect of cis-dichlorodiammineplatinum (II) on the surface of tumor and normal cells. Ind. J. Exp. Biol. 19:328 1981.

68. Ghadially F.N., Lock C.J., Lalonde J.M., et. al., Platinosomes produced in synovial membrane by platinum coordination complexes. Virchow Arch. B. Cell. Path. 35:123 1981.

69. Berry J.P., Brille P., LeRoy A.F., et. al., Experimental ultrastructural and X-ray microanalysis study of cisplatin in the rat: intracellular localization of platinum. Cancer Treat. Rep. 66:1529 1982.

70. Heinen F., Effects of antimitotic agents either free or bound to DNA on mouse macrophages cultivated in vitro. Virchow Arch. B. Cell. Path. 27:79 1978.

71. Ghadially F.N., Lock C.J., Yang-Steppuhn S.E., et. al., Platinosomes produced in cultured cells by platinum coordination complexes. J. Submicrosc. Cytol. 13:223 1981.

72. Khan M.U., Sadler P.J., Distribution of platinum anti-tumor drug in Hela cells by analytical electron microscopy. Chem. Biol. Interact. 21:227 1978.

73. Hoeschele J.D., Johnson N.P., Rahn R.O., et. al., Comparative distribution/binding studies of ^{195m}Pt-labeled dichlorodiammineplatinum (II), DDP, in the rat with emphasis on the localization in DNA. Biochimie 60:1054 1978.

74. Choie D.D., Del Campo A.A., Guarino A.M., Subcellular localization of cis-dichlorodiammineplatinum (II) in rat kidney and liver. Toxicol. Appl. Pharmacol. 55:245 1980.

75. Dickerson R.E., Eisenberg D., Varnum J., et. al., $PtCl_4^{-2}$: A methionine-specific label for protein crystallography. J. Mol. Biol. 45:77 1969.

76. Melius P., Teggins J.E., Friedman M.E., et. al., Inhibition of leucine aminopeptidase and malate dehydrogenase by aquoplatinum (II) complexes. Biochim. Biophys. Acta 268:194 1972.

77. Friedman M.E., Musgrove B., Lee K., Inhibition of malate dehydrogenase by platinum (II) complexes. Biochim. Biophys. Acta 250:288 1971.

78. Teggins J.E., Friedman M.E., The inhibition of malate dehydrogenase by chloroamine-platinum (II) complexes. Biochim. Biophys. Acta 350:273 1974.

79. Friedman M.E., Melius P., McAuliffe C.A., A proposed mechanism relating the antitumor behavior of cis-platinum amine complexes to their inhibition of a model enzyme. Bioinorg. Chem. 8:355 1978.

80. Friedman M.E., Melius P., Teggins J.E., et. al., Interactions of cis- and trans- platinum (II) complexes with dehydrogenase enzymes in the presence of mono- and polynucleotides: evidence for a ternary complex. Bioinorg. Chem. 8:341 1978.

81. Friedman M.E., Melius P., Interaction of platinum (II) complexes with several oxidoreductases in the presence or absence of nucleic acids. J. Clin. Hematol. Oncol. 7:503 1977.

82. Daley-Yates P.T., McBrien D.C., The inhibition of renal ATPase by cisplatin and some biotransformation products. Chem. Biol. Interact. 40:325 1982.

83. Aull J.L., Allen R.L., Bapat A.R., et. al., The effects of platinum complexes on seven enzymes. Biochim. Biophys. Acta 571:352 1979.

84. Friedman M.E., Teggins J.E., The reactivities of isomers of dichlorodiamineplatinum (II) with dehydrogenase enzymes. Evidence for inhibition via cross-linkage. Biochim. Biophys. Acta 350:263 1974.

85. Allen R.C., Gale G.R., Oulla P.M., et. al., Effects of certain antitumor platinum compounds on kidney esterases. Bioinorg. Chem. 8:83 1978.

86. Aull J.L., Rice A.C., Tebbetts L.A., Interactions of platinum complexes with the essential and nonessential sulphydryl groups of thymidylate synthetase. Biochemistry 16:672 1977.

87. Hall I.H., Holshouser M.H., Loeffler L.J., Effects of cis-malonato-diammino platinum (II) on P-388 lymphocytic leukemia cell metabolism. J. Pharm. Sci. 69:1160 1980.

88. Giraldi T., Taylor D.M., The effects of platinum ethylenediamine dichloride on the template activity of DNA. Biochem. Pharmacol. 23:1659 1974.

89. Harder H.C., Smith R.G., LeRoy A.F., Template primer inactivation by cis- and trans- dichlorodiammine platinum for human DNA polymerase alpha, beta, and rauscher murine leukemia virus reverse transcriptase, as a mechanism of cytotoxicity. Cancer Res. 36:3821 1976.

90. Johnson N.P., Hoeschele J.D., Kuemmerle N.B., Effects of platinum antitumor agents and pyrimidine dimers on the in vitro replication of T7 DNA. Chem. Biol. Interact. 23:267 1978.

91. Srivastava R.C., Froehlich J., Eichhorn G.L., The effect of platinum binding on the structure of DNA and its function in RNA synthesis. Biochimie 60:879 1978.

92. Kelman A.D., Peresie H.J., Stone P.J., An analysis of the modes of binding of antitumor platinum complexes to DNA. J. Clin. Hematol. Oncol. 7:440 1977.

93. Mansy S., Rosenberg B., The interaction of platinum compounds with nucleosides and nucleic acids, in "Advances in Antimicrobial and Antineoplastic Chemotherapy", Vol. 2, M. Semonsky, M. Hejzlar, S. Masak, eds., University Park Press, Baltimore, 1972, pp. 191-2.

94. Millard M.M., Macquet J.P., Theophanides T., X-ray photoelectron spectroscopy of DNA · Pt complexes. Evidence of O^6(Gua) · N_7 (Gua) chelation of DNA with cis-dichlorodiammine platinum (II). Biochim. Biophys. Acta 402:166 1975.

95. Chu G.Y., Tobias R.S., Heavy metal-nucleotide interactions. 8. Binding of cis-diammineplatinum (II) and ethylenediammineplatinum (II) to inosine, 1-methylinosine, and 5'-inosine monophosphate in aqueous solution. J. Am. Chem. Soc. 98:2641 1976.

96. Mansey S., Rosenberg B., Thompson A.J., Binding of cis-and trans-dichlorodiammineplatinum (II) to nucleosides. I. Location of binding sites. J. Am. Chem. Soc. 95:1633 1973.

97. Nee M., Roberts J.D., Studies of the binding interactions of cis-diamminedichloroplatinum (II) with amines and nucleosides by nitrogen-15 nuclear magnetic resonance. Biochemistry 21:4920 1982.

98. Goodgame D.M., Jeeves I., Phillips F.L., et. al., Possible mode of action of antitumor platinum drugs: X-ray evidence for cis binding by platinum of two inosine 5'-monophosphate molecules via the N(7) positions. Biochim. Biophys. Acta 378:153 1975.

99. Roos I.A., Thomson A.J., Mansy S., Interaction of platinum compounds with dinucleotides. J. Am. Chem. Soc. 96:6484 1974.

100. Girault J.P., Chottard G., Lallemand J-Y., et. al., Interaction of $[Pt(NH_3)_2(H_2O)_2](NO_3)_2$ with ribose and deoxyribose diguanine phosphates. Biochemistry 21:1352 1982.

101. Marcelis A.T., den Hartog J.H., Reedjik J., Intrastrand cross-linking of the guanines of the deoxytrinucleotide (G-C-G) via cis-$Pt(NH_3)_2Cl_2$. J. Am. Chem. Soc. 104:2664 1982.

102. Stone P.J., Kelman A.D., Sinex F.M., Resolution of alpha, beta, and gamma DNA of Saccharomyces cerevisiae with the antitumor drug cis-$Pt(NH_3)_2Cl_2$. Evidence for preferential drug binding by GpG sequences in DNA. J. Mol. Biol. 104:793 1976.

103. Barton J.K., Szalda D.J., Rabinowitz H.N., et. al., Solid state structure, magnetic susceptibility, and single crystal ESR properties of cis-diammineplatinum and pyridone blue. J. Am. Chem. Soc. 101:1434 1979.

104. Barton J.K., Caravana C., Lippard S.J., Chemical and spectroscopic characterization of cis-diammineplatinum and alpha pyridone blue in aqueous solution. Comparison with other platinum blues. J. Am. Chem. Soc. 101:7269 1979.

105. Laurent J.P., LePage P., Castan P., et. al., Platinum blues. Experimental support for the occurrence of platinum (III) in a blue compound involving 1-methylnicotinamide and guanosine ligands. Inorg. Chim. Acta 67:31 1982.

106. Flynn C.M., Viswanathan T.S., Martin R.B., The constitution of platinum blues. J. Inorg. Nucl. Chem. 39:437 1977.

107. Davidson J.P., Faber P.J., Fischer R.G., et. al., Platinum-pyrimidine blues and related complexes: a new class of potent antitumor agents. Cancer Chemother. Rep. 59:287 1975.

108. Lippert B., Platinum pyrimidine blues - nature and possible structure. J. Clin. Hematol. Oncol. 7:26 1977.

109. Cleare M.J., Chemistry of co-ordination complexes. Rec. Results Cancer Res. 48:12 1974.

110. Robins A.B., The reaction of ^{14}C-labelled platinum ethlene-diamine dichloride with nucleic acid constituents. Chem. Biol. Interact. 6:35 1973.

111. Shooter K.V., Howse R., Merrifield R.K., et. al., The interaction of platinum II compounds with bacteriophages T7 and R17. Chem. Biol. Interact. 5:289 1972.

112. Louie S., Bau R., Structure of $[Pt(en)(5'\text{-CMP})]_2 \cdot 2H_2O$. An example of direct platinum-phosphate bonding. J. Am. Chem. Soc. 99:3874 1977.

113. Jack A., Ladner J.E., Rhodes D., A crystallographic study of metal binding to yeast phenylalanine transfer RNA. J. Mol. Biol. 111:315 1977.

114. Eshaque M., McKay M.J., Theophanides T., D-mannitol platinum complexes. J. Clin. Hematol. Oncol. 7:338 1977.

115. Drobnik J., Krekulova A., Kubelkova A., Inactivation of bacteriophages with cis-platinum (II) diamminodichloride. Folia Microbiol. 16:548 1971.

116. Resolva S., The induction of lysogenic strains of Escherichia coli by cis-dichlorodiammineplatinum (II). Chem. Biol. Interact. 4:66 1971.

117. Köpf-Maier P., Wagner W., Liss E., Cytokinetic behaviour of Ehrlich ascites tumor after in vivo treatment with cis-diamminedichloroplatinum (II) and metallocene dichlorides. J. Cancer Res. Clin. Oncol. 201:21 1981.

118. Levine B.S., Preache M.M., Pergament E., Mutagenic potential of cis-dichlorodiammine platinum II in rodents. Toxicol. 17:57 1980.

119. Benedict W.F., Baker M.S., Haroun L., et. al., Mutagenicity of cancer chemotherapeutic agents in the Salmonella/microsome test. Cancer Res. 37:2209 1977.

120. Harder H.C., Rosenberg B., Inhibitory effects of antitumor platinum compounds on DNA, RNA and protein syntheses in mammalian cells in vitro. Int. J. Cancer 6:207 1970.

121. Howle J.A., Gale G.R., Cis-dichlorodiammeplatinum (II). Persistent and selective inhibition of deoxyribonucleic acid synthesis in vivo. Biochem. Pharmacol. 19:2757 1970.

122. Howle J.A., Thompson H.S., Stone A.E., et. al., Cis-dichlorodiammineplatinum (II): inhibition of nucleic acid synthesis in lymphocytes stimulated with phytohemagglutinin. Proc. Soc. Exp. Biol. Med. 137:820 1971.

123. Erickson L.C., Zwelling L.A., Ducore J.M., et. al., Differential cytotoxicity and DNA cross-linking in normal and transformed human fibroblasts treated with cis-diamminedichloroplatinum (II). Cancer Res. 41:2791 1981.

124. Pascoe J.M., Roberts J.J., Interactions between mammalian cell DNA and inorganic platinum compounds I. DNA interstrand cross-linking and cytotoxic properties of platinum (II) compounds. Biochem. Pharmacol. 23:1345 1974.

125. Stone P.J., Kelman A.D., Sinex F.M., Specific binding of anti-tumor drug cis-$Pt(NH_3)_2Cl_2$ to DNA rich in guanine and cytosine. Nature 251:736 1974.

126. Munchausen L.L., Rahn R.O., Biologic and chemical effects of cis-dichlorodiammineplatinum (II) (NSC-119875) on DNA. Cancer Chemother. Rep. 59:643 1975.

127. Tullius T.D., Lippard S.J., cis-diamminedichloroplatinum (II) binds in a unique manner to oligo(dG)·oligo(dC) sequences in DNA - A new assay using exonuclease III. J. Am. Chem. Soc. 103:4620 1981.

128. Horăcek P., Drobnik J., Interaction of cis-dichlorodiammine-platinum (II) with DNA. Biochim. Biophys. Acta 254:341 1971.

129. Roberts J.J., Pascoe J.M., Cross-linking of complimentary strands of DNA in mammalian cells by antitumor platinum compounds. Nature 235:282 1972.

130. Zwelling L.A., Micheals S., Schwartz H., et. al., DNA cross-linking as an indicator of sensitivity and resistance of mouse L1210 leukemia to cis-diamminedichloroplatinum (II) and L-phenylalanine mustard. Cancer Res. 41:640 1980.

131. Laurent G., Erickson L.C., Sharkey N.A., et. al., DNA cross-linking and cytotoxicity induced by cis-diamminedichloroplatinum (II) in human normal and tumor cell lines. Cancer Res. 41:3347 1981.

132. Zwelling L.A., Anderson T., Kohn K.W., DNA-protein and DNA interstrand cross-linking by cis- and trans- platinum (II) diamminedichloride in L1210 mouse leukemia cells and relation to cytotoxicity. Cancer Res. 39:365 1979.

133. Ducore J.M., Erickson L.C., Zwelling L.A., et. al., Comparative studies of DNA cross-linking and cytotoxicity in Burkitt's lymphoma cell lines treated with cis-diamminedichloroplatinum (II) and L-phenylalanine mustard. Cancer Res. 42:897 1982.

134. Strandberg M.C., Bresnick E., Eastman A., The significance of DNA cross-linking to cis-diamminedichloroplatinum (II) - induced cytotoxicity in sensitive and resistant lines of murine leukemia L1210 cells. Chem. Biol. Interact. 39:169 1982.

135. Zwelling L.A., Bradley M.V., Sharkey N.A., et. al., Mutagenicity, cytotoxicity and DNA cross-linking in V79 chinese hamster cells treated with cis- and trans- Pt(II) diamminedichloride. Mutat. Res. 67:271 1979.

136. Macquet J.P., Butour J.L., Modifications of the DNA secondary structure upon platinum binding: a proposed model. Biochimie 60:901 1978.

137. Roberts J.J., Friedlos F., The frequency of interstrand cross-links in DNA following reaction of cis-diamminedichloroplatinum (II) with cells in culture or DNA in vitro: stability of DNA cross-links and their repair. Chem. Biol. Interact. 39:181 1982.

138. Roberts J.J., Friedlos F., Quantitative aspects of the formation and loss of DNA interstrand cross-links in chinese hamster cells following treatment with cis-diamminedichloroplatinum (II) (Cisplatin). I. Proportion of DNA - platinum reactions involved with cross-linking. Biochim. Biophys. Acta 655:146 1981.

139. Pera M.F., Rawlings C.J., Shakelton J., et. al., Quantitative aspects of the formation and loss of DNA interstrand cross-links in chinese hamster cells following treatment with cis-diamminedichloroplatinum (II) (Cisplatin). II. Comparison of results from alkaline elution, DNA renaturation and DNA sedimentation studies. Biochim. Biophys. Acta 655:153 1981.

140. Monti-Bragadin C., Tamaro M., Banfi E., Mutagenic activity of platinum and ruthenium complexes. Chem. Biol. Interact. 11:469 1975.

141. Roos I.A., The interaction of an anti-tumor platinum complex with DNA. Chem. Biol. Interact. 16:39 1977.

142. Macquet J.P., Butour J.L., DNA-platinum interactions. Flourescence studies and DNA saturation with cis-$Pt(NH_3)_2Cl_2$. J. Clin. Hematol. Oncol. 7:469 1977.

143. Butour J.L., Macquet J.P., Differentiation of DNA · platinum complexes by flourescence. The use of an intercalating dye as a probe. Eur. J. Biochem. 78:455 1977.

144. Cohen G.L., Bauer W.R., Barton J.K., et. al., Binding of cis- and trans- dichlorodiammineplatinum (II) to DNA: evidence for unwinding and shortening of the double helix. Science 203:1014 1979.

145. Mong S., Daskal Y., Prestayko A., et. al., DNA supercoiling, shortening and induction of single-stranded regions by cis-diamminedichloroplatinum (II). Cancer Res. 41:4020 1981.

146. Scovell W.M., Kross L.R., Cis- and trans- diamminedichloroplatinum (II) binding produces different tertiary structural changes in SV40 DNA. Biochem. Biophys. Res. Comm. 104:1597 1982.

147. Cohen G.L., Ledner J.A., Bauer W.R., et. al., Sequence dependent binding of cis-dichlorodiammineplatinum (II) to DNA. J. Am. Chem. Soc. 102:2487 1980.

148. Macquet J.P., Butour J.L., A circular dichroism study of DNA·platinum complexes. Eur. J. Biochem. 83:375 1978.

149. Macquet J.P., Theophanides T., DNA-platinum interactions in vitro with trans- and cis- $Pt(NH_3)_2Cl_2$. Bioinorg. Chem. 5:59 1975.

150. Cramer R.E., Dahlstrom P.L., The crystal and molecular structure of cis $[Pt(NH_3)_2(Guo)_2Cl_{3/2}(ClO)_4)_{1/2} \cdot 7H_2O$. J. Clin. Hematol. Oncol. 7:440 1977.

151. Kong P., Theophanides T., Reactions of cis $[Pt(NH_3)_2Cl_2]$ and cis$[Pt(en)Cl_2]$ with nucleosides. Inorg. Chem. 13:1167 1974.

152. Marcelis A.T., van Kralingen C.G., Reedijk J., The interactions of cis- and trans- diammineplatinum compounds with 5'-guanosine monophosphate and 5'-deoxyguanosine monophosphate. A proton n.m.r. investigation. J. Inorg. Biochem. 13:213 1980.

153. Gellert R.W., Bau R., The structure of the [platinum(ethylenediammine)(guanosine)$_2$]$^{2+}$ cation. J. Am. Chem. Soc. 97:7379 1979.

154. Bau R., Gellert R.W., Lehovec S.M., et. al., Crystallographic studies on platinum-nucleoside and platinum-nucleotide complexes. J. Clin. Hematol. Oncol. 7:51 1977.

155. Carsey T.P., Boudreaux E.A., The electronic structure of platinum-guanine complexes. Chem. Biol. Interact. 30:189 1980.

156. Boudreaux E.A., Carsey T.P., Quasirelativistic MO calculations on platinum complexes (anticancer drugs) and their interaction with DNA. Int. J. Quantum Chem. 18:469 1980.

157. Scovell W.M., O'Connor T., Interaction of aquated cis-[$(NH_3)_2$ Pt(II)] with nucleic acid constituents. 1. Ribonucleosides. J. Am. Chem. Soc. 99:120 1977.

158. O'Connor T., Scovell W.M., The interaction of aquated cis-[$(NH_3)_2$ Pt(II)] nucleic acids constituents. II. Modified nucleosides. Chem. Biol. Interact. 26:227 1979.

159. Royer-Pokora B., Gordon K.L., Haseltine W.A., Use of exonuclease III to determine the site of stable lesions in defined sequences of DNA: the cyclobutane pyrimidine dimer and cis and trans dichlorodiammine platinum (II) examples. Nucl. Acid Res. 9:4595 1981.

160. Fichtinger-Schepman A.M., Lohman P.H., Reedijk J., Detection and quantification of adducts formed upon the interaction of diamminedichloroplatinum (II) with DNA, by anion-exchange chromatography after enzymatic degradation. Nucl. Acid Res. 10:5345 1982.

161. Jordanov J., Williams R.J., Interaction of the antitumor drug cis-$Pt(NH_3)_2Cl_2$ with cytidilyl-3'-5'-guanosine. Bioinorg. Chem. 8:77 1978.

162. Inagaki K., Kidani Y., Complex of cis-$Pt(NH_3)_2Cl_2$ with guanylyl (3'-5')-cytidine. J. Inorg. Biochem. 11:39 1979.

163. Konishi H., Usui T., Sawada H., et. al., Effects of anticancer platinum compounds on Escherichia coli strains with normal and defective DNA repair capacity. Gann 72:627 1981.

164. Drobnik J., Urbenkova M., Krekulova A., The effect of cis-dichlorodiammine-platinum II on Escherichia coli B, the role of fil, exr, and hcr markers. Mutat. Res. 17:13 1973.

165. Beck D.J., Brubaker R.R., Effect of cis-platinum (II) diamminedichloride on wild type and DNA repair deficient mutants of E. coli. J. Bacteriol. 116:1247 1973.

166. Fraval H.N., Rawlings C.J., Roberts J.J., Increased sensitivity of UV repair-deficient human cells to DNA bound platinum products which unlike thymine dimers are not recognized by an endonuclease extracted from Micrococcus luteus. Mutat. Res. 51:121 1978.

167. Fraval H.N., Roberts J.J., Excision repair of cis-diamminedichloroplatinum (II)-induced damage to DNA of chinese hamster cells. Cancer Res. 39:1793 1979.

168. Van den Berg H.W., Roberts J.J., Investigations into the mechanism of action of antitumor platinum compounds: time and dose dependent changes in the alkaline sucrose gradient sedimentation profiles of DNA from hamster cells treated with cis-platinum (II) diamminedichloride. Chem. Biol. Interact. 11:493 1975.

169. Zwelling L.A., Kohn K.W., Ross W.E., et. al., Kinetics of formation and disappearance of a DNA cross-linking effect in mouse leukemia L1210 cells treated with cis- and trans-diamminedichloroplatinum (II). Cancer Res. 38:1762 1978.

170. Pera M.F., Rawlings C.J., Roberts J.J., The role of DNA repair in the recovery of human cells from cisplatin toxicity. Chem. Biol. Interact. 37:245 1981.

171. Roberts J.J., Fraval H.N., The interaction of antitumor platinum compounds with cellular DNA in cultured cells and animal tissues: relationship to DNA cellular repair processes. Biochimie 60:869 1978.

172. Alazard R.J., Germanier M., Post replication repair in an excision defective strain on Escherichia coli following treatment with cis-dichlorodiammineplatinum (II). Biochem. Biophys. Res. Comm. 104:693 1982.

173. Van den Berg H.W., Fraval H.N., Roberts J.J., Repair of DNA damaged by neutral platinum compounds. J. Clin. Hematol. Oncol. 7:349 1977.

174. Van den Berg H.W., Roberts J.J., Inhibition by caffeine of post-replication repair in chinese hamster cells treated with cis-platinum diamminedichloride: the extent of platinum binding to the template DNA in plation to the size of low molecular weight nascent DNA. Chem. Biol. Interact. 12:375 1976.

175. Kovarik J., Svec F., Thurzo V., The effect of cis-dichlorodiammineplatinum (II) and acronycin on the proliferation and respiration of Hela cells in vitro. Neoplasma 19:569 1972.

176. Gosalen M., Blanco M., Hunter J., et. al., Effects of anticancer agents on the respiration of isolated mitochondria and tumor cells. Eur. J. Cancer 10:567 1974.

177. Van den Berg H.W., Roberts J.J., Post-replication repair of DNA in chinese hamster cells treated with cis-platinum (II) diamminedichloride. Enhancement of toxicity and chromosome damage by caffeine. Mutat. Res. 33:279 1975.

178. Beck D.J., Fisch J.E., Mutagenicity of platinum coordination complexes in Salmonella typhimurium. Mutat. Res. 77:45 1980.

179. LeCointe P., Macquet J.P., Butour J.L., et. al., Relative efficiencies of a series of square-planar platinum (II) compounds on Salmonella mutagenesis. Mutat. Res. 48:139 1977.

180. Beck D.J., Brubaker R.R., Mutagenic properties of cis-platinum (II) diamminodichloride in Escherichia Coli. Mutat. Res. 27:181 1975.

181. Brouwer J., Van de Putte P., Fichtinger-Schepman A.M., et. al., Base-pair substitution hotspots in GAG and GCG nucleotide sequences in E. Coli K12, induced by cis-diamminodichloroplatinum (II). Proc. Natl. Acad. Sci. (U.S.A.) 78:7010 1981.

182. Cocchialla L., Mattern I.E., Van Kralingen C.G., Mutagenicity of antitumor platinum compounds. Mutat. Res. 74:252 1980.

183. Beck D.J., Vazquez D., Cook R., Effect of cis-platinum (II) diamminodichloride on wild type and A rec A mutant of Escherichia coli. J. Clin. Hematol. Oncol. 7:726 1977.

184. Sogaard-Anderson K., Platinum (II) complexes generate frameshift mutations in test strains of Salmonella typhimurium. Mutat. Res. 67:209 1979.

185. Mattern I.E., Cocchiarella L., van Kralingen C.G., et. al., Prophage induction and mutagenicity of a series of anti-tumor platinum (II) and platinum (IV) co-ordination complexes. Mutat. Res. 95:79 1982.

186. Turnbull D., Popescu N.C., DiPaolo J.A., et. al., Cis-platinum diammine dichloride causes mutation, transformation, and sister-chromatid exchanges in cultured mammalian cells. Mutat. Res. 66:267 1971.

187. Morrison W.D., Huff V., Colyer S.P., et. al., Cytogenic effects of cisplatinum (II) diamminedichloride in vivo. Environ. Mutagenesis 3:265 1981.

188. Wiencke J.K., Cervenka J., Paulis H., Mutagenic activity of anticancer agent cis-dichlorodiammineplatinum (II). Mutat. Res. 68:69 1979.

189. Khan A., Hill J.M., Immunosuppression with cis-platinum (II) diamminodichloride: effect on plaque-forming spleen cells. Infect. Immun. 4:320 1971.

190. Khan A., Hill J.M., Suppression of graft-versus-host reaction by cis-platinum (II) diamminodichloride. Transplantation 13:55 1972.

191. Khan A., Hill J.M., Inhibition of lymphocyte blastogenesis in patients receiving cis-platinous diamminodichloride. Proc. Am. Assoc. Cancer Res. 13:92 1972.

192. Khan A., Albayrak A., Hill J.M., Effect of cis-platinous diamminodichloride on graft rejection: prolonged survival of skin grafts against H_2 histocompatability. Proc. Soc. Exp. Biol. Med. 141:7 1972.

193. Brambilla G., Cavanna M., Maura A., Effect of cis-diamminedichloroplatinum (NSC-119875) on the allograft reaction in mice. Cancer Chemother. Rep. 58:633 1974.

194. Fumarulo R., Giordano D., Riccardi S., et. al., Modification of macrophage chemotaxis caused by cis-$Pt(NH_3)_2Cl_2$. Proc. Soc. Exp. Biol. Med. 164:164 1980.

195. Munster A.M., Greenberg P., Greenberg S., et. al., Effect of cis-platinum (II) diamminedichloride and gallium nitrate on the ability of spleen cells to induce a graft-versus-host reaction. Proc. Soc. Exp. Biol. Med. 146:333 1974.

196. Presnov M.A., Konovalova A.L., Romanova L.F., et. al., Chemotherapy of transplantable mouse tumors with cis-dichlorodiammine-platinum (II) alone and in combination with sarcolysin. Cancer Treat. Rep. 62:705 1978.

197. Sodhi A., Aggarwal S.K., Effects of cis-dichlorodiammine platinum (II) in the regression of sarcoma 180: a fine structural study. J. Natl. Cancer Inst. 53:85 1974.

198. Conran P.B., Rosenberg B., The role of host defense in the regression of sarcoma-180 in mice treated cis-dichlorodiammine-platinum, in "Advances in Antimicrobial and Antimeoplastic Chemotherapy", Vol. 2, M. Semonsky, M. Hejzlar, S. Masak, eds., University Park Press, Baltimore, 1972, pp. 235-6.

199. Mally M.B., Taylor R.C., Callewaert D.M., Effects of platinum antitumor agents on in vitro assays of human antitumor immunity II. Effects of cis-$Pt(NH_3)_2Cl_2$ on spontaneous cell-mediated cytotoxicity. Chemotherapy 26:1 1980.

200. Kleinerman E.S., Zwelling L.A., Muchmore A.V., Enhancement of naturally occuring human spontaneous monocyte-mediated cytotoxicity by cis-diamminedichloroplatinum (II). Cancer Res. 40:3099 1980.

201. McAllister P.K., Rosenberg B., Aggarwal S.K., et. al., Electron microscopic studies of mammalian cells stained with platinum-pyrimidine blues. J. Clin. Hematol. Oncol. 7:717 1977.

202. HaLL D.J., Diasio R., Goplerud D.R., Cis-platinum in gynecologic cancer. I. Epithelial ovarian cancer. Am. J. Obstet. Gynecol. 141:299 1981.

203. Bruckner H.W., Cohen C.J., Goldberg J.D., et. al., Improved chemotherapy for ovarian cancer with cis-diamminedichloroplatinum and adriamycin. Cancer 47:2288 1981.

204. Bruckner H.W., Cohen C.J., Deppe G., et. al., Effect of cis-diamminedichloroplatinum chemotherapy on the survival of patients with advanced ovarian cancer. Mt. Sinai J. Med. (N.Y.) 48:121 1981.

205. Barker G.H., Wiltshaw E., Use of high dose cis-dichlorodiammine platinum (II) (NSC-119875) following failure on previous chemotherapy for advanced carcinoma of the ovary. Br. J. Obstet. Gynecol. 88:1192 1981.

206. Wittes R., Heller K., Randolph V., Cis-dichlorodiammineplatinum-based cancer chemotherapy in the initial treatment of advanced head and neck cancer. Cancer Treat. Rep. 63:1533 1979.

207. Kish J., Drelichman A., Jacobs J., et. al., Clinical trial of cisplatin and 5-FU infusion as initial treatment for advanced squamous cell carcinoma of the head and neck. Cancer Treat. Rep. 66:471 1982.

208. Coker D.D., Elias E.G., Chretien P.B., et. al., Combination chemotherapy for advanced squamous cell carcinoma of the head and neck. Head Neck Surg. 4:111 1981.

209. Baker S.R., Makuch R.W., Wolf G.T., Preoperative cisplatin and bleomycin therapy in head and neck squamous cell carcinoma. Prognostic factors for tumor response. Arch. Otolaryngol. 107:683 1981.

210. Mendenhall W.L., Willaims S.D., Einhorn L.H., et. al., Disseminated seminoma: re-evaluation of treatment protocols. J. Urol. 126:493 1981.

211. Einhorn L.H., Williams S.D., Chemotherapy of disseminated seminoma. Cancer Clin. Trials 3:307 1980.

212. Kelsen D.P., Bains M., Hilaris B., et. al., Combination chemotherapy of esophageal carcinoma using cisplatin, vindesine, and bleomycin. Cancer 49:1174 1982.

213. Panettiere F.J., Leichman L., O'Bryan R., et. al., cis-diammine-dichloride platinum (II), an effective agent in the treatment of epidermoid carcinoma of the esophagus. A preliminary report of an ongoing southwest oncology group study. Cancer Clin. Trials 4:29 1981.

214. Vogl S.E., Greenwald E., Kaplan B.H., Effective chemotherapy for esophageal cancer with methotrexate, bleomycin, and cis-diamminedichloroplatinum (II). Cancer 48:2555 1981.

215. Thigpen T., Shingleton H., Homesley H., et. al., Cis-platinum in the treatment of advanced or recurrent squamous cell carcinoma of the cervix: a phase II study of the gynecologic oncology group. Cancer 48:899 1981.

216. Hall D.J., Diasio R., Goplerud D.R., Cis-platinum in gynecologic cancer. II. Squamous cell carcinoma of the cervix. Am. J. Obstet. Gynecol. 141:305 1981.

217. Alberts D.S., Martimbeau P.W., Surwit E.A., et. al., Mitomycin-C, bleomycin, vincristine and cis-platinum in the treatment of advanced, recurrent squamous cell carcinoma of the cervix. Cancer Clin. Trials 4:267 1981.

218. Kedia K.R., Gibbons C., Persky L., The management of advanced bladder carcinoma. J. Urol. 125:655 1981.

219. Yagoda A., Phase II trials with cis-dichlorodiammineplatinum (II) in the treatment of urothelial cancer. Cancer Treat. Rep. 63:1565 1979.

220. Carter S.K., Wasserman T.H., The chemotherapy of urologic cancer. Cancer 36:729 1975.

221. Bhuchar V.K., Lanzotti V.J., High-dose cis-platin for lung cancer. Cancer Treat. Rep. 66:375 1982.

222. Davis S., Rambotti P., Park Y.K., Combination cyclophosphamide, doxorubicin, and cisplatin (CAP) chemotherapy for extensive non-small cell carcinomas of the lung. Cancer Treat. Rep. 65:955 1981.

223. Eagan R.T., Ingle J.N., Frytak S., et. al., Platinum-based poly-chemotherapy versus dianhydrogalactitol in advanced non-small cell lung cancer. Cancer Treat. Rep. 61:1339 1977.

224. Kelsen D., Gralla R., Stoopler M., et. al., Cisplatin, doxorubicin, cyclophosphamide, and vindesine combination chemotherapy for non-small cell lung cancer. Cancer Treat. Rep. 66:247 1982.

225. Takita H., Edgerton F., Marabella P., et. al., Platinum-based combination chemotherapy in non-small cell lung carcinoma. Cancer 48:1528 1981.

226. Zak M., Drobnik J., Effect of cis-dichlorodiammineplatinum (II) on the post-irradiation lethality in mice after irradiations with X-rays. Strahlentherapie 142:112 1971.

227. Wodinsky I., Swiniarski J., Kensler C.J., et. al., Combination radiotherapy and chemotherapy for P388 lymphocytic leukemia in vivo. Cancer Treat. Rep. 4:73 1974.

228. Richmond R.C., Powers E.L., Radiation sensitization of bacterial spores by cis-dichlorodiammineplatinum (II). Rad. Res. 68:25 1976.

229. Zimbrick J.D., Sukrochana A., Richmond R.C., Studies on radiosensitization of Escherichia coli cells by cis-platinum complexes. Int. J. Rad. Oncol. Biol. Phys. 5:1351 1979.

230. Richmond R.C., Zimbrick J.D., Hykes D.L., Radiation-induced DNA damage and lethality in E. coli as modified by the antitumor agent cis-dichlorodiammineplatinum (II). Rad. Res. 71:447 1977.

231. Douple E.B., Richmond R.C., Logon M.E., Therapeutic potention in a mouse mammary tumor and an intracerebral rat brain tumor by combined treatment with cis-dichloro-diammineplatinum (II) and radiation. J. Clin. Hematol. Oncol. 7:585 1977.

232. Szumiel I., Requirements for potentiation of radiation effect by a platinum complex. Int. J. Rad. Biol. 33:605 1978.

233. Chadwick K.H., Leenhouts H.P., Szumiel I., et. al., An analysis of the interaction of a platinum complex and radiation with CHO cells using the molecular theory of cell survival. Int. J. Radiat. Biol. 30:511 1976.

234. Douple E.B., Richmond R.C., Platinum complexes as radiosensitizers of hypoxic mammalian cells. Br. J. Cancer 37:98 1978 (Suppl. III).

235. Muggia F.M., Glatstein E., Summary of investigations on platinum compounds and radiation interactions. Int. J. Rad. Oncol. Biol. Phys. 5:1407 1979.

236. Porschen W., Muhlensiepen H., Porschen R., et. al., Combined action of ionizing radiation and cis-dichlorodiammineplatinum (II) on a solid tumor: external measurements and isobologram analysis. (in press).

237. Douple E.B., Richmond R.C., A review of platinum complex biochemistry suggests a rationale for combined platinum-radiotherapy. Int. J. Rad. Oncol. Biol. Phys. 5:1335 1979.

238. Burholt D.R. Schenken L.L., Kovacs C.J., et. al., Response of the murine gastrointestinal epithelium to cis-dichlorodiammineplatinum (II). Int. J. Rad. Oncol. Biol. Phys. 5:1377 1979.

239. Greene R.F., Chatterji D.C., Hiranka P.K., et. al., Stability of cisplatin in aqueous solution. Am. J. Hosp. Pharm. 36:38 1979.

240. Litterest C.L., Alterations in the toxicity of cis-dichlorodiammineplatinum (II) and in tissue localization of platinum as a function of NaCl concentration in the vehicle of administration. Toxicol. Appl. Pharmacol. 61:99 1981.

241. Mavligit G.M., Benjamin R., Patt Y.Z., et. al., Intraarterial cis-platinum for patients with inoperable skeletal tumors. Cancer 48:1 1981.

242. Lokich J.L., Phase I study of cis-diamminedichloroplatinum (II) administered as a constant 5-day infusion. Cancer Treat. Rep. 64:905 1980.

243. Reddel R.R., Kefford R.F., Grant J.M., et. al., Ototoxicity in patients receiving cisplatin: importance of dose and method of administration. Cancer Treat. Rep. 66:19 1982.

244. Pretorius R.G., Petrilli E.S., Kean C., Comparison of the IV and IP routes of administration of cisplatin in dogs. Cancer Treat. Rep. 65:1055 1981.

245. Drewinko B., Gottlieb J.A., Action of cis-dichlorodiammineplatinum (II) (NSC-119875) at the cellular level. Cancer Chemother. Rep. 59:665 1975.

246. Bergerat J.P., Barlogie B., Drewinko B., Effects of cis-dichlorodiammineplatinum (II) on human colon carcinoma cells in vitro. Cancer Res. 39:1334 1979.

247. Moran R.E., Straus M.J., Effects of pulse and continuous intravenous infusion of cis-diamminedichloroplatinum on L1210 leukemia in vivo. Cancer Res. 41:4993 1981.

248. Ostrow S., Egorin M.J., Hahn D., et. al., High dose cisplatin therapy using mannitol versus furosemide diuresis: Comparative pharmacokinetics and toxicity. Cancer Treat. Rep. 65:73 1981.

249. Nitschke R., Fagundo R., Berry D.H., Weekly administration of cis-dichlorodiammineplatinum (II) in childhood solid tumors: a southwest oncology group study. Cancer Treat. Rep. 63:497 1979.

250. Ostrow S., Egorin M., Hahn D., High dose cis-dichlorodiammine platinum (C-DDP) therapy: pharmacokinetic analysis and toxicity using furosemide (F) vs. mannitol (M) diuresis. Proc. Am. Assoc. Cancer Res. 20:88 1979.

251. Vogl S.E., Zaravinos T., Kaplan B.H., et. al., Safe and effective two hour outpatient regimen of hydration and diuresis for the administration of cis-diamminedichloroplatinum (II). Eur. J. Cancer 17:345 1981.

252. Deliconstantinos G., Gregoriadis G., Abel G., et. al., Incorporation of cis-dichlorobiscyclopentyl-ammineplatinum (II) into liposomes enhances its uptake by ADJ/PC 6A tumors implanted subcutaneously into mice. Biochem. Soc. Trans. 5:1326 1977.

253. Yatvin M.B., Muehlensiepen H., Porschen W., et. al., Selective delivery of liposome-associated cis-dichlorodiammineplatinum (II) by heat and its influence on tumor uptake and growth. Cancer Res. 41:1602 1981.

254. Friese J., Mueller W.H., Magerstedt P., et. al., Pharmacokinetics of liposome encapsulated cisplatin in rats. Arch. Int. Pharmacodyn. 258:180 1982.

255. Wenzel M., Schneider M., Bier J., Toxicity and cytostatic effects of $Pt(NH_3)_2Cl_2$ after i.v. and i.t. injections. Z. Naturforsch 35:848 1980.

256. Schaeppi U., Heyman I.A., Fleischman R.W., et. al., Cis-dichlorodiammineplatinum (II) (NSC-119875): preclinical toxicologic evaluation of intravenous injection in dogs, monkeys and mice. Toxicol. Appl. Pharmacol. 25:230 1973.

257. Dixon R.L., Cooney D.A., Davis R.D., et. al., Preclinical toxicological evaluation of cis-dichlorodiammineplatinum (II), in "Advances in Antimicrobial and Antineoplastic Chemotherapy", Vol. 2., M. Semonsky, M. Hejzlar, S. Masak, eds., University Park Press, Baltimore, 1972, pp. 243-5.

258. Choie D.D., Longdecker D.S., Copley M.P., Cytotoxicity of cis-platin in rat intestine. Toxicol. Appl. Pharmocol. 60:354 1981.

259. Kociba R.J., Sleight S.D., Acute toxicologic and pathologic effects of cis-diamminedichloroplatinum (NSC-119875) in the male rat. Cancer Chemother. Rep. 55:1 1971.

260. Khan T., Elias E.G., Mason G.R., A single dose of metoclopramide in the control of vomiting from cis-dichlorodiammineplatinum (II) in man. Cancer Treat. Rep. 62:1106 1978.

261. Gylys J.A., Doran K.M., Buyniski J.P., Antagonism of cisplatin induced emesis in the dog. Res. Comm. Chem. Pathol. Pharmacol. 23:61 1979.

262. Staquet M., Bron D., Rozencweig M., et. al., Clinical studies with a THC analog [BRL-4664] in the prevention of cis-platin-induced vomiting. J. Clin. Pharmacol. 21:605 1981.

263. McCarthy L.E., Borison H.L., Antiemetic activity of N-methyll-evonantradol and nabilone in cisplatin-treated cats. J. Clin. Pharmacol. 21:305 1981.

264. Wilson T., Weltz M., Solimando D., et. al., Coninuous infusion of droperidol: anti-emetic therapy for cis-platinum toxicity. Proc. Am. Soc. Cancer Res. 22:351 1981.

265. Grossman B., Lessin L.S., Cohen P., Droperidol prevents nausea and vomiting from cis-platinum. N. Engl. J. Med. 301:47 1980.

266. Robins I.H., Ershler W.B., DeJongh L., et. al., Antiemetic effect of intravenous diazepam in patients receiving cis-diamminedi-chloroplatinum II. A pilot study. Pediatr. Oncol. 7:247 1979.

267. Florcysk A.P., Schurig J.E., Bradner W.T., Cisplatin-induced emesis in the ferret: a new animal model. Cancer Treat. Rep. 66:187 1982.

268. Strum S.B., Opfell R.W., McDermed J.E., et. al., Intravenous metoclopramide (MCP) inhibition of cis-platinum (DDP) induced nausea and vomiting. Proc. Am. Soc. Cancer Res. 22:231 1981.

269. Gralla R.J., Squillante A., Itri L.M., et. al., High-dose meto-clopramide: effective antiemetic against cis-platin in randomized trials vs placebo and prochlorperazine. Proc. Am. Soc. Cancer Res. 22:344 1981.

270. Frytak S., Moertel C.G., Eagan R.T., et. al., A double-blind comparison of metoclopramide and prochloperazine as antiemetics for platinum therapy. Proc. Am. Soc. Cancer Res. 22:350 1981.

271. Drapkin R., McAloon E., Sokol G., et. al., The antiemetic effect of dexamethasone in patients receiving cis-platinum. Proc. Am. Soc. Cancer Res. 22:340 1981.

272. Aapro M.S., Alberts D.S., High-dose dexamethasone for prevention of cis-platin-induced vomiting. Cancer Chemother. Pharmacol. 7:11 1981.

273. Mason B.A., Dambra J., Grossman B., et. al., Effective control of cisplatin-induced nausea using high-dose steroids and droperidol. Cancer Treat. Rep. 66:243 1982.

274. Curry S.L., Rine J., Whitney C.W., et. al., Role of prostaglandins in the excessive nausea and vomiting after intravascular cis-platinum therapy. Gynecol. Oncol. 12:89 1981.

275. Giudicelli C.P., Ricordel I., Jospovici J.J., et. al., Kinetic study of cis-diamminedichloroplatinum in a case of serous cystadenocarcinoma of ovary with severe renal insufficiency. Thérapie 36:653 1981.

276. Zakharova I.A., Tomilets V.A., Dontsov V.I., Histamine-liberating and histamine binding action of platinum and palladium compounds. Inorg. Chim. Acta 46:L3 1980.

277. Cowan J.D., Kies M.S., Roth J.L., et. al., Nerve conduction studies in patients treated with cis-diamminedichloroplatinum (II): a preliminary report. Cancer Treat. Rep. 64:1119 1980.

278. Reinstein L., Ostrow S.S., Wiernik P.H., Serial nerve conduction studies in cis-platinum (II) (C-DDP) peripheral neuropathy. Arch. Phys. Med. Rehabil. 59:558 1978.

279. Von Hoff D.D., Reichert C.M., Cuneo R., et. al., Demyelination of peripheral nerves associated with cis-diamminedichloroplatinum II therapy. Proc. Am. Assoc. Cancer Res. 20:91 1979.

280. Kedar A., Cohen M.E., Freeman A.I., Peripheral neuropathy as a complication of cis-diamminedichloroplatinum (II) treatment: a case report. Cancer Treat. Rep. 62:819 1978.

281. Von Hoff D.D., Schilsky R., Reichert C.M., et. al., Toxic effects of cis-dichlorodiammineplatinum (II) in man. Cancer Treat. Rep. 63:1527 1979.

282. Helson L., Okonkwo E., Anton L., et. al., Cis-platinum ototoxicity. Clin. Toxicol. 13:469 1978.

283. Piel I.J., Meyer D., Perlia C.P., et. al., Effects of cis-diamminedichloroplatinum (NSC-119875) on hearing function in man. Cancer Chemother. Rep. 58:871 1974.

284. Aguilar-Markulis N.V., Beckley S., Priore R., et. al., Auditory toxicity effects of long-term cis-dichlorodiammineplatinum II therapy in genitourinary cancer patients. J. Surg. Oncol. 16:111 1981.

285. Nakai Y., Konishi K., Chang K.C., et. al., Ototoxicity of the anticancer drug cisplatin. An experimental study. Acta Otolaryngol. 93:227 1982.

286. Komune S., Snow J.B., Potentiating effects of cis-platin and ethacrynic acid in ototoxicity. Arch. Otolaryngol. 107:594 1981.

287. Stadnicki S.W., Fleischman R.W., Schaeppi U., et. al., Cis-dichlorodiammineplatinum (II) (NSC-119875): hearing loss and other toxic effects in rhesus monkeys. Cancer Chemother. Rep. 59:467 1975.

288. Fleischman R.W., Stadnicki S.W., Ethier M.F., et. al., Ototoxicity of cis-dichlorodiammine platinum (II) in the guinea pig. Toxicol. Appl. Pharmacol. 33:320 1975.

289. Komune S., Asakuma S., Snow J.B., Pathophysiology of the ototoxicity of cis-diamminedichloroplatinum. Otolaryngol. Head Neck Surg. 89:275 1981.

290. Talley R.W., O'Bryan R.M., Gutterman J.U., et. al., Clinical evaluation of toxic effects of cis-diamminedichloroplatinum (NSC-119875) - phase I clinical study. Cancer Chemother. Rep. 57:465 1973.

291. Corder M.P., Elliot T.E., Bell S.J., Dose limiting myelotoxicity in absence of significant nephrotoxicity with a weekly out-patient schedule of cis-platinum (II) diamminedichloride. J. Clin. Hematol. Oncol. 7:645 1977.

292. Van Nguyen B., Jaffe N., Cisplatin-induced anemia. Cancer Treat. Rep. 65:1121 1981.

293. Getaz E.P., Beckley S., Fitzpatrick J., et. al., Cisplatin-induced hemolysis. N. Engl. J. Med. 302:334 1980.

294. Kuzur M.E., Greco F.A., Cis-platin-induced anemia. N. Engl. J. Med. 303:110 1980.

295. Najarian T., Miller A., Zimelman A.P., et. al., Hematologic effect of cis-platinum-bleomycin therapy. Oncology 38:195 1981.

296. Jenkins V.K., Perry R.R., Goodrich W.E., Effects of cis-diamminedichloroplatinum (II) on hematopoietic stem cells in mice. Exp. Hematol. 9:281 1981.

297. Ogawa M., Gale G.R., Kurn S.S., Effect of cis-diamminedichloroplatinum (NSC-119875) on murine and human hemopoietic precursor cells. Cancer Res. 35:1398 1975.

298. Fishman P., Levi J., Notti I., et. al., Synthesizing activity and ultrastructural findings of human blood cells after incubation with cis-platinum in vitro. Biomedicine 34:78 1981.

299. Thompson H.S., Gale G.R., cis-dichlorodiammineplatinum (II): Hematopoietic effects in rats. Toxicol. Appl. Pharmacol. 19:602 1971.

300. Walker E.M., Gale G.R., Methods of reduction of cisplatin nephrotoxicity. Ann. Clin. Lab. Sci. 11:397 1981.

301. Jones B.R., Bhalla R.B., Mladek J., et. al., Comparison of methods of evaluating nephrotoxicity of cis-platinum. Clin. Pharmacol. Therap. 27:557 1980.

302. Kuhn J.A., Angy W.P., Rakowski T.A., et. al., Nephrotoxicity of cis-diamminedichloroplatinum (II) as measured by urinary beta-glucuronidase. Cancer Treat. Rep. 64:1083 1980.

303. Goldstein R.S., Noordewier B., Bond J.T., et. al., Cis-dichloro-diammineplatinum nephrotoxicity: time course and dose response of renal functional impairment. Toxicol. Appl. Pharmacol. 60:163 1981.

304. Groth S., Nielson H., Pederson A.G., Nephrotoxicity of cis-platinum in man. A method for early detection. Proc. 3rd World Congress of Nucelar Medicine and Biology, C. Raynaud, ed., Pergamon Press, Paris, 1982, pp. 580-583.

305. Ward J.M., Fauvie K.A., The nephrotoxic effects of cis-diammine-dichloroplatinum (II) (NSC-119875) in male F344 rats. Toxicol. Appl. Pharmacol. 38:535 1976.

306. Dobyan D.C., Hill D., Lewis T., et. al., Cyst formation in rat kidney induced by cis-platinum administration. Lab. Invest. 45:260 1981.

307. Levi J., Jacobs C., Kalman S., et. al., Mechanism of cis-platinum nephrotoxicity: I. Effects of sulfhydryl groups in rat kidneys. J. Pharmacol. Exp. Therap. 213:545 1980.

308. Dobyan D.C., Levi J., Jacobs C., et. al., Mechanism of cis-platinum nephrotoxicity: II. Morphologic observations. J. Pharmacol. Exp. Therap. 213:551 1980.

309. Gongalez-Vitale J.C., Hayes D.M., Cvitkovic E., et. al., The renal pathology in clinical trials of cis-platinum (II) diamminedichloride. Cancer 39:1362 1977.

310. Choie D.D., Longnecker D.S., Del Campo A.A., Acute and chronic cisplatin nephrotoxicity in rats. Lab. Invest. 44:397 1981.

311. Lyman N.W., Hemalatha C., Viscuso R.L., et. al., Cisplatin-induced hypocalcemia and hypomagnesemia. Arch. Int. Med. 140:1513 1980.

312. Schilsky R.L., Anderson T., Hypomagnesemia and renal magnesium wasting in patients receiving cis-platin. Ann. Intern. Med. 90:929 1979.

313. Hayes F.A., Green A.A., Senzer N., et. al., Tetany: a complication of cis-dichlorodiammineplatinum (II) therapy. Cancer Treat. Rep. 63:547 1979.

314. Guarino A.M., Miller D.S., Arnold S.T., et. al., Platinate toxicity: past, present, and prospects. Cancer Treat. Rep. 63:1475 1979.

315. Nitschke R., Renal complications of cis-diamminedichloroplatinum. Ann. Clin. Lab. Sci. 11:392 1981.

316. Hayes D., Cvitkovic E., Golbey R., Amelioration of renal toxicity of high dose cis-platinum diammine dichloride (CPDD) by mannitol induced diuresis. Proc. Am. Assoc. Cancer Res. 17:169 1976.

317. Ward J.M., Grabin M.E., LeRoy A.F., et. al., Modification of the renal toxicity of cis-dichlorodiammineplatinum (II) with furosemide in male F344 rats. Cancer Treat. Rep. 61:379 1977.

318. Pera M.F. Jr., Harder H.C., Effects of mannitol and furosemide diuresis on cis-dichlorodiammineplatinum (II) antitumor activity and toxicity to host-renewing cell populations in rats. Cancer Res. 39:1279 1979.

319. Ward J.M., Grabin M.E., Berlin E., et. al., Prevention of renal failure in rats receiving cis-diamminedichloroplatinum (II) by administration of furosemide. Cancer Res. 37:1238 1977.

320. Pera M.F., Harder H.C., Effects of furosemide (FM) - and mannitol (MN) - induced diuresis on the nephrotoxicity and physiologic disposition of cis-dichlorodiammineplatinum (CDDP) in rats. Proc. Am. Assoc. Cancer Res. 19:100 1978.

321. Lehane D., Winston A., Gray R., et. al., The effects of diuretic pretreatment on clinical, morphological, and ultrastructural cis-platinum induced nephrotoxicity. Int. J. Radiat. Oncol. Biol. Phys. 5:1393 1979.

322. Hayes D.M., Cvitkovic E., Golbey R.M., High dose cis-platinum diamminedichloride: amelioration of renal toxicity by mannitol diuresis. Cancer 39:1372 1977.

323. Stark J.J., Howell S.B., Carmody J., Prevention of cis-platinum nephrotoxicity. Clin. Res. 25:412 1977.

324. Belt R.J., Taylor S., Randomized trial of hydration and mannitol (Mn) in preventing cis-platinum (CDDP) induced nephrotoxicity. Proc. Am. Assoc. Cancer Res. 20:388 1979.

325. Al-Sarraf M., Clinical trial of cis-platinum (NSC-119875): hydration with or without mannitol in patients with previously treated advanced malignant melanoma: a southwest oncology group study. Proc. Am. Assoc. Cancer Res. 20:185 1979.

326. Stark J.J., Howell S.B., Nephrotoxicity of cis-platinum (II) - dichlorodiammine. Clin. Pharmacol. Ther. 23:461 1978.

327. Burchenal J.H., Kalaher K., Dew K., et. al., Studies of cross-resistance, synergistic combinations and blocking activity of platinum derivatives. Biochimie 60:961 1978.

328. Filipski J., Kohn K.W., Prather R., et. al., Thiourea reverses cross-links and restores biological activity in DNA treated with dichlorodiammineplatinum (II). Science 204:181 1979.

329. Ishizawa M., Taniguchi S., Baba T., Protection by sodium thiosulphate and thiourea against lethal toxicity of cis-diamminedichloroplatinum (II) in bacteria and mice. Japan. J. Pharmacol. 31:883 1981.

330. Yuhas J.M., Culo F., Selective inhibition of the nephrotoxicity of cis-dichlorodiammineplatinum (II). Cancer Treat. Rep. 64:57 1980.

331. Howell S.B., Taetle R., Effect of thiosulphate on cis-dichlorodiammineplatinum (II) toxicity and antitumor activity in L1210 leukemia. Cancer Treat. Rep. 64:611 1980.

332. Borch R.F., Katz J.C., Lieder P.H., et. al., Effect of diethyldithiocarbamate rescue on tumor response to cis-platinum in a rat model. Proc. Natl. Acad. Sci. 77:5441 1980.

333. Krakoff I., Nephrotoxicity of cis-dichlorodiammineplatinum (II). Cancer Treat. Rep. 63:1523 1979.

334. Slater T.F., Ahmed M., Ibrahim S.A., Studies on the nephrotoxicity of cis-dichlorodiammine-platinum^{2+} and related substances. J. Clin. Hematol. Oncol. 7:534 1977.

335. Borch R.F., Pleasants M.E., Inhibition of cis-platinum nephrotoxicity by diethyldithiocarbamate rescue in a rat model. Proc. Natl. Acad. Sci. 76:6611 1979.

336. Gale G.R., Atkins L.M., Cis-platin and diethyldithiocarbamate in the treatment of L1210 leukemia. J. Clin. Hematol. Oncol. 11:41 1981.

337. Ross D.A., Gale G.R., Reduction of the renal toxicity of cis-dichlorodiammineplatinum (II) by probenecid. Cancer Treat. Rep. 63:781 1979.

338. Broomhead J.A., Fairlie D.P., Whitehouse M.W., cis-platinum (II) amine complexes: Some structure-activity relationships for immunosuppressive, nephrotoxic and gastrointestinal (side) effects in rats. Chem. Biol. Interact. 31:113 1980.

339. McGuiness J.E., Proctor P., Demopoulos P.H., et. al., Amelioration of cis-platinum nephrotoxicity by orgotein (superoxide dismutase). Physiol. Chem. Phys. 10:267 1978.

340. Bertolero F., Litterest C.L., Changes in renal handling of platinum in cis-platinum-treated rats following induction of metabolic acidosis or alkalosis. Res. Comm. Chem. Pathol. Pharmacol. 36:273 1982.

341. Levi F.A., Hrushesky W.J., Blomquist C.H., et. al., Reduction of cis-diamminedichloroplatinum nephrotoxicity in rats by optimal circadian drug timing. Cancer Res. 42:950 1982.

342. Hrushesky W.J., Levi F.A., Halberg F., et. al., Circadian stage dependence of cis-diamminedichloroplatinum lethal toxicity in rats. Cancer Res. 42:945 1982.

343. Sheka I.A., Chaus I.S., Mityureva T.T., "The Chemistry of Gallium", Elsevier Publishing Co., Amsterdam, 1966, pp. 1-20, 28-57.

344. Dudley H.C., Maddox G.E., La Rue H.C., Studies of the metabolism of gallium. J. Pharmacol. Exp. Therap. 96:135 1949.

345. Dudley H.C., Munn J.I., Henry K.E., Studies of the metabolism of gallium. II. J. Pharmacol. Exp. Therap. 98:105 1950.

346. Munn J.I., Walters N.H., Dudley H.C., Urinary excretion of gallium by man and animals. J. Lab. Clin. Med. 37:676 1951.

347. Dudley H.C., Marrer H.H., Studies of the metabolism of gallium. III. Deposition in and clearance from bone. J. Pharmacol. Exp. Therap. 106:129 1952.

348. Edwards C.L., Hayes R.L., Tumor scanning with ^{67}Ga-citrate. J. Nucl. Med. 10:103 1969.

349. Edwards C.L., Hayes R.L., Scanning malignant neoplasms with gallium-67. J. Am. Med. Assoc. 212:1182 1970.

350. Lederer C.M., Shirley V.S., eds., "Table of the Isotopes", 7th edition, John Wiley and Sons, New York, N.Y., pp. 212-3.

351. Silvester D.J., Thakur M.L., Cyclotron production of carrier-free gallium-67. Int. J. Appl. Radiat. Isot. 21:630 1970.

352. Gruverman I.J., Kruger P., Cyclotron-produced carrier-free radioisotopes. Int. J. Appl. Radiat. Isot. 5:21 1959.

353. Brown L.C., Callahan A.P., Skidmore M.R., et. al., High yield Zn-68 cyclotron targets for gallium-67 production. Int. J. Appl. Radiat. Isot. 24:651 1973.

354. Neirinckx R.D., A high yield production method for gallium-67 using an electroplated natural zinc or enriched Zn-66 target. Int. J. Appl. Radiat. Isot. 27:1 1976.

355. Nass H.W., Accepted gallium-67 decay data. J. Nucl. Med. 21:704 1980.

356. New England Nuclear, Gallium-67 citrate, Package insert.

357. Dahl J.R., Tilbury R.S., The use of a compact, multiparticle cyclotron for the production of ^{59}Fe, ^{67}Ga, ^{111}In, ^{123}I for medical purposes. Int. J. Appl. Radiat. Isot. 23:431 1972.

358. Steyn J., Meyer B.R., Production of ^{67}Ga by deuteron bombardment of natural zinc. Int. J. Appl. Radiat. Isot. 24:369 1973.

359. Porter J., Kawana M., Krizek H., et. al., ^{67}Ga production with a compact cyclotron. J. Nucl. Med. 11:352 1970.

360. Hupf H.B., Beaver J.E., Cyclotron production of ^{67}Ga for medical applications. J. Nucl. Med. 10:343 1969.

361. Dymov A.M., Savostin A.P., "Analytical Chemistry of Gallium", Ann Arbor Science Publishers, London, 1970, pp. 1-22.

362. Wade K., Bannister A.J., Chapter 12:Al, Ga, In, and Tl, in "Comprehensive Inorganic Chemistry", A.F. Trotman-Dickenson exec. editor, Pergamon Press, Oxford, 1973, p. 1094.

363. Kulprathipanja S., Hnatowich D.J., Evans G., The effect of pH on the *in vitro* and *in vivo* behavior of complex-free ^{68}Ga and ^{113m}In. Int. J. Nucl. Med. Biol. 5:140 1978.

364. Lavrova G.V., Tsimmergakl V.A., Infared absorption spectra of citratocomplexes of gallium and indium. Russ. J. Inorg. Chem. 12:486 1967.

365. Kulba F.Y., Babkina N.A., Zharkov A.P., Potentiometric investigation of complex formation by gallium (III) with citric acid. Russ. J. Inorg. Chem. 20:1461 1975.

366. Blanco R.E., Perkinson, J.D., Preparation of gallium citrate by ion exchange. J. Am. Chem. Soc. 73:2696 1951.

367. Sheka I.A., Lavrova G.V., Complexing of gallium with citric acid. Chem. Abst. 49179 1971.

368. Harris W.R., Martell A.E., Aqueous complexes of gallium (III). Inorg. Chem. 15:713 1976.

369. Chang C.H., Pitner T.P., Lenkinski R.E., et. al., Carbon-13 fourier transform nuclear magnetic resonance study of gallium citrate in aqueous solution. J. Am. Chem. Soc. 99:5858 1977.

370. Glickson J.D., Pitner T.P., Webb J., et. al., Hydrogen-1 and gallium-71 nuclear magnetic resonance study of gallium citrate in aqueous solution. J. Am. Chem. Soc. 97:1679 1975.

371. Hnatowich D.J., Kulprathipanja S., Beh R., The importance of pH and citrate concentration on the *in vitro* and *in vivo* behavior of radiogallium. Int. J. Appl. Rad. Isot. 28:925 1977.

372. Waxman A.D., Kawada T., Wolf W., et. al., Are all gallium citrate preparations the same? Radiology 117:647 1975.

373. Glaubitt D., Kaul A., Koeppe P., et. al., Kinetic studies in rats for the determination of radiation dose by ^{67}Ga, in "Proceedings of the Second Congress of the European Association of Radiology". Excerpta Medica, Amsterdam, 1971, pp. 535-541.

374. Johnston G.S., Gallium-67 imaging in clinical medicine, New England Nuclear Teaching Monograph, 1976.

375. Small R.C., Bennett L.R., Normal ^{67}Ga scan. J. Nucl. Med. 12:394 1971.

376. Larson S.M., Hoffer P.B., Normal patterns of localization, in "Gallium-67 Imaging" P.B. Hoffer, C. Bekerman, R. Henkin, eds., J. Wiley & Sons, Toronto, 1978, pp. 23-28.

377. Edwards C.L., Hayes R.L., Gallium-67 citrate for tumor scanning, in "Radiologic and Other Biophysical Methods in Tumor Diagnosis", Year Book Medical Publishers Inc., Chicago, 1973 pp. 39-56.

378. Nelson B., Hayes R.L., Edwards C.L., et. al., Distribution of gallium in human tissues after intravenous administration. J. Nucl. Med. 13:92 1972.

379. Edwards C.L., Hayes R.L., Localization of tumors with radioisotopes, in "Clinical Uses of Radionuclides: Critical Comparison with Other Techniques", F.A. Goswitz, G.A. Andrews, M. Viamonte, eds., U.S. Atomic Energy Commission Office of Information Services, Springfield, 1972, pp. 618-639.

380. Logan K.J., Ng P.K., Turner C.J., et. al., Comparative pharmacokinetics of ^{67}Ga and ^{59}Fe in humans. Int. J. Nucl. Med. Biol. 8:271 1981.

381. Clausen J., Edeling C.J., Fogh J., ^{67}Ga binding to human serum proteins and tumor components. Cancer Res. 34:1931 1974.

382. Konikowski T., Glenn H.J., Haynie T.P., Kinetics of ^{67}Ga compounds in brain sarcomas and kidneys of mice. J. Nucl. Med. 14:164 1973.

383. Sephton R.G., Hodgson G.S., DeAbrew S., et. al., Ga-67 and Fe-59 distributions in mice. J. Nucl. Med. 19:930 1978.

384. Hartman R.E., Hayes R.L., The binding of gallium by blood serum. J. Pharmacol. Exp. Therap. 168:193 1969.

385. Hartman R.E., Hayes R.L., Indium and gallium binding by blood serum. Fed. Proc. 27:838 1968.

386. Gunasekera S.W., King L.J., Lavender P.J., The behavior of tracer gallium-67 towards serum proteins. Clin. Chim. Acta 39:401 1972.

387. Hara T., On the binding of gallium to transferrin. J. Nucl. Med. Biol. 1:152 1974.

388. Vallabhajosula S.R., Harwig J.F., Sieman J.K., et. al., Radiogallium localization in tumors: blood binding and transport and the role of transferrin. J. Nucl. Med. 21:650 1980.

389. Harris D.C., Gray G.A., Aisen P., ^{13}C nuclear magnetic resonance study of the spatial resolution of the metal and anion binding sites of human transferrin. J. Biol. Chem. 249:5261 1974.

390. Rasey J.S., Larson S.M., Nelson N.J., Mechanism of gallium-67 uptake by tumors: similarities of iron and gallium metabolism. J. Nucl. Med. 21:P23 1980.

391. Hayes R.L., Byrd B.L., Rafter J.J., et. al., The effect of scandium on the tissue distribution of Ga-67 in normal and tumor-bearing rodents. J. Nucl. Med. 21:361 1980.

392. Hammersley P.A., Taylor D.M., The effect of the administration of iron on gallium 67 citrate uptake in tumors. Br. J. Radiol. 53:563 1980.

393. Watson E.E., Cloutier R.J., Gibbs W.B., Whole body retention of ^{67}Ga citrate. J. Nucl. Med. 14:840 1973.

394. Hammersley P.A., Zivanovic M.A., The relationship of ^{67}Ga uptake to citrate dose, compared with ^{67}Ga-chloride and ^{67}Ga- transferrin in rodent tissues and tumor. Nukl. Med. 19:25 1980.

395. Mallet-Paret S., The influence of BCG on the distribution of radiogallium. M.Sc. Thesis, University of Alberta, 1980.

396. Zivanovic M.A., McCready V.R., Taylor D.M., The urinary excretion of gallium-67 citrate in patients with neoplastic disease. Eur. J. Nucl. Med. 4:277 1979.

397. Zivanovic M.A., Taylor D.M., McCready V.R., The chemical form of gallium-67 in urine. Int. J. Nucl. Med. Biol. 5:97 1978.

398. Gallé P., Mechanism of renal concentration and elimination of indium and gallium: a study by nuclear tomography at the microscopic level. Eur. J. Nucl. Med. 6:A14 1981.

399. Green A., Gallium citrate-Ga-67. New England Nuclear department reference, 1976.

400. Taylor A., Chafetz N., Hollenbeck J., et. al., The source of fecal gallium-clinical implications. J. Nucl. Med. 19:1214 1978.

401. Chen D.C., Scheffel U., Camargo E.E., et. al., The source of gallium-67 gastrointestinal contents. J. Nucl. Med. 21:1146 1980.

402. Newstead G., Taylor D.M., McCready V.R., et. al., Gallium-67 deposition in the human gastrointestinal tract. Int. J. Nucl. Med. Biol. 4:109 1977.

403. Dalrymple G.V., Body C.M., Moss A.J., et. al., Metabolism of ^{67}Ga by mammalian cells in culture. J. Nucl. Med. 12:349 1971.

404. Swartzendruber D.C., Byrd B.L., Hayes R.L., et. al., Preferential localization of gallium-67 citrate in tissue of leukemic mice. J. Natl. Cancer Inst. 44:695 1970.

405. Bandurski M., Pollock R.J., Rosenberg B., et. al., The interactions of anticancer salts of group IIIA elements with biomacromolecules. J. Clin. Hematol. Oncol. 7:411 1977.

406. Brown D.H., Swartzendruber D.C., Carlton J.E., et. al., The isolation and characterization of gallium-binding granules from soft tissue tumors. Cancer Res. 33:2063 1973.

407. Aulbert E., Haubold U., Isolation of the ^{67}Ga accumulating fraction in normal rat liver. Nukl. Med. 13:72 1974.

408. Takeda S., Uchida T., Matsuzowa T., A comparative study on lysosomal accumulation of gallium-67 and indium-111 in Morris hepatoma 7316A. J. Nucl. Med. 18:835 1977.

409. Takeda S., Okuyama S., Takusagawa K., et. al., Lysosomal accumulation of gallium-67 in Morris hepatoma-7316A and Shionogi mammary carcinoma-115. Gann 69:267 1978.

410. Hammersley P.A., Taylor D.M., The role of lysosomal enzyme activity in the localization of ^{67}gallium citrate. Eur. J. Nucl. Med. 4:261 1979.

411. Aulbert E., Gebhardt A., Schulz E., et. al., Mechanism of ^{67}Ga accumulation in normal rat liver lysosomes. Nucl. Med. 15:185 1976.

412. Bekerman C., Vyas M.J., Renal localization of ^{67}Ga-citrate in renal amyloidosis: case reports. J. Nucl. Med. 17:899 1976.

413. Orii H., Tumor scanning with gallium (^{67}Ga) and its mechanism studies in rats. Strahlentherapie 144:192 1972.

414. Ito Y., Okuyama S., Sato K., et. al., ^{67}Ga tumor scanning and its mechanisms studied in rabbits. Radiology 100:357 1971.

415. Ando A., Ando I., Takeshita M., Subcellular distribution of gallium-67 in tumor and liver. Int. J. Nucl. Med. Biol. 9:65 1981.

416. Hayes R.L., Brown D.H., Biokinetics of radiogallium, in "Nuklearmedizin, Fortschritte der Nuklearmedizin in Klinischer und Technologischer Sicht", W.B. Pabst, G. Hor, H.A. Schmidt, eds., Schautauer Verlag, New York, 1975, pp. 837-848.

417. Brown D.H., Byrd B.L., Carlton J.E., et. al., A quantitative study of the subcellular localization of ^{67}Ga. Cancer Res. 36:956 1976.

418. Swartzendruber D.C., Nelson B., Hayes R.L., Gallium-67 localization in lysosomal-like granules of leukemic and non-leukemic murine tissues. J. Natl. Cancer Inst. 46:941 1971.

419. Samezima K., Orii H., In vivo localization of ^{67}Ga-citrate in rat liver as demonstrated by cell fractionation with isopycnic rate-zonal centrifugation. Eur. J. Nucl. Med. 5:281 1980.

420. Pierrez J., Bertrand A., The fixation of gallium. J. Nucl. Med. 20:456 1979.

421. Hayes R.L., Carlton J.E., A study of the macromolecular binding of ^{67}Ga in normal and malignant animal tissues. Cancer Res. 33:3272 1973.

422. Lawless D., Brown D.H., Hubner K.F., Isolation and partial characterization of a ^{67}Ga-binding glycoprotein from Morris 5123C rat hepatoma. Cancer Res. 38:4440 1978.

423. Larson S., Grunbaum Z., Rasey J.S., The role of transferrins in gallium uptake. Int. J. Nucl. Med. Biol. 8:257 1981.

424. Lentle B.C., Garrecht B., Schmidt R., et. al., A difference between patients with and without tumor uptake of radiogallium. Int. J. Nucl. Med. Biol. 8:267 1981.

425. Hegge F.N., Mahler D.J., Larson S.M., The incorporation of Ga-67 into the ferritin fraction of rabbit hepatocytes in vivo. J. Nucl. Med. 18:937 1977.

426. Samezima K., Nakamura K., Orii H., The role of ferritin in the in vivo localization of gallium-67. Int. J. Nucl. Med. Biol. 8:94 1981.

427. Hoffer P.B., Huberty J., Khayam-Bashi H., The association of Ga-67 and lactoferrin. J. Nucl. Med. 18:713 1977.

428. Mason D.Y., Taylor C.R., Distribution of transferrin, ferritin and lactoferrin in human tissues. J. Clin. Pathol. 31:316 1978.

429. de Vet B.J., Tenhoopen C.H., Lactoferrin in human neutrophilic polymorphonuclear leukocytes in relation to iron metabolism. Acta Med. Scand. 203:197 1978.

430. Hoffer P.B., Miller-Catchpole R., Turner D.A., Demonstration of lactoferrin in tumor tissue from patients with gallium scans. J. Nucl. Med. 20:424 1979.

431. Hoffer P.B., Imaging technique, in "Gallium-67 Imaging", P.B. Hoffer, C. Bekerman, R.E. Henkin, eds., John Wiley and Sons, Toronto, 1978, p. 10-21.

432. Hoffer P.B., Schor R., Ashby D., et. al., Comparison of Ga-67 citrate images obtained with rectilinear scanner and large field of view Anger camera. J. Nucl. Med. 18:538 1977.

433. Silberstein E.B., Fernandez-Olloa M., Hall J., Are oral cathartics of value in optimizing the gallium scan? J. Nucl. Med. 22:424 1981.

434. Zeman R.K., Ryerson T.W., The value of bowel preparation in Ga-67 citrate scanning. J. Nucl. Med. 18:886 1977.

435. Novetsky G.J., Turner D.A., Ali A.A., et. al., Cleansing the colon in gallium-67 scintigraphy: a prospective comparison of regimens. Am. J. Roentgenol. 137:979 1981.

436. Hoffer P.B., Status of gallium-67 in tumor detection. J. Nucl. Med. 21:394 1980.

437. Yang S.L., Alderson P.O., Kaizer H.A., et. al., Serial ^{67}Ga-citrate imaging in children with neoplastic disease. J. Nucl. Med. 20:210 1979.

438. Yeh S.D., Benua R.S., Tan C.T., Gallium scanning in recurrent Hodgkin's disease in children. Clin. Nucl. Med. 4:359 1979.

439. Johnston G.S., Go M.F., Benua R.S., et. al., Gallium-67 citrate imaging in Hodgkin's disease: final report of a cooperative group. J. Nucl. Med. 18:692 1977.

440. Martin J.J., Sephton R.G., Gallium-67 scanning in malignant lymphoma. Aust. Radiol. 22:340 1978.

441. Huys J., Schelstraete K., Simons M., Ga-67 imaging in Hodgkin's disease. Clin. Nucl. Med. 7:174 1982.

442. Longo D.L., Schilsky R.L., Blei L., et. al., Gallium-67 scanning: limited usefulness in staging patients with non-Hodgkin's lymphoma. Am. J. Med. 68:695 1980.

443. Andrews G.A., Hubner K.F., Greenlaw R.H., Ga-67 citrate imaging in malignant lymphoma: final report of a cooperative group. J. Nucl. Med. 19:1013 1978.

444. Brown M.L., O'Donnell J.B., Thrall J.H., et. al., Gallium-67 scintigraphy in untreated and treated non-Hodgkin's lymphomas. J. Nucl. Med. 19:875 1978.

445. Cabanillas F., Zornoza J., Haynie T.P., et. al., Comparison of lymphangiograms and gallium scans in the non-Hodgkin's lymphomas. Cancer 39:85 1977.

446. Richman S.D., Appelbaum F., Levenson S.M., et. al., ^{67}Ga radionuclide imaging in Burkitt's lymphoma. Radiology 117:639 1975.

447. Arseneau J.C., Aamodt R., Johnston G.S., et. al., Evidence for granulocytic incorporation of ^{67}gallium in chronic granulocytic leukemia. J. Lab. Clin. Med. 83:496 1974.

448. Milder S., Glick J.H., Henderson E.S., et. al., ^{67}Ga scintigraphy in acute leukemia. Cancer 32:803 1973.

449. Bekerman C., Hoffer P.B., Other malignancies in which gallium-67 is useful: hepatoma, melanoma, and leukemia, in "Gallium-67 Imaging", P.B. Hoffer, C. Berkerman, R.E. Henkin, eds., John Wiley and Sons, New York, 1978, pp. 153-160.

450. Milder M.S., Frankel R.S., Bulkly G.B., et. al., Gallium-67 scintigraphy in malignant melanoma. Cancer 32:1350 1973.

451. Jackson F.I., McPherson T.A., Lentle B.C., Gallium-67 scintigraphy in multisystem malignant melanoma. Radiology 122:163 1977.

452. Richman S.D., Ingle J.N., Levenson S.M., et. al., Usefulness of gallium scintigraphy in primary and metastatic breast carcinoma. J. Nucl. Med. 16:996 1975.

453. Oyamada H., Kawai H., Fukukita H., et. al., Subtraction scintigraphy with ^{67}Ga-citrate and ^{99m}Tc-colloid in the diagnosis of intrahepatic masses. Radioisotopes 29:14 1980.

454. Hauser M.F., Alderson P.V., Gallium-67 imaging in abdominal disease. Sem. Nucl. Med. 8:251 1978.

455. Waxman A.D., Richmond R., Juttner H., et.al., Correlation of contrast angiography and histologic pattern with gallium uptake in primary liver cell carcinoma: non-correlation with alpha-fetoprotein. J. Nucl. Med. 21:324 1980.

456. Suzuki T., Matsumoto Y., Manabe T., et. al., Serum alpha-fetoprotein and Ga^{67} citrate uptake in hepatoma. Am. J. Roentgen. Rad. Ther. Nucl. Med. 120:627 1974.

457. Frankel R.S., Jones A.E., Chen J.A., et. al., Clinical correlations of ^{67}Ga and skeletal whole body radionuclide studies with radiography in Ewings sarcoma. Radiology 110:597 1974.

458. Teates C.D., Bray S.T., Williamson B.R., Tumor detection with ^{67}Ga-citrate: a literature survey (1970-1978). Clin. Nucl. Med. 3:456 1978.

459. Bailey T.B., Pinskey S.M., Mittemeyer B.T., et. al., A new adjunct in testis tumor staging: gallium-67 citrate. J. Urol. 110:307 1973.

460. Paterson A.H., Peckham M.J., McCready V.R., Value of gallium scanning in seminoma of the testis. Br. Med. J. 1:118 1976.

461. Bloomfield R.D., Wilfrido M.S., Yoon T., et. al., The use of ^{67}Ga citrate in gynecologic malignancies. Gynecol. Oncol. 6:130 1978.

462. Jackson F.I., Dierich H.C., Lentle B.C., Gallium-67 citrate scintiscanning in testicular neoplasia. J. Can. Assoc. Radiol. 27:84 1976.

463. Saverbrunn B.J., Andrews G.A., Hubner K.F., Ga-67 citrate imaging in tumors of the genito-urinary tract: report of cooperative study. J. Nucl. Med. 19:470 1978.

464. Douds H.N., Berens S.V., Long R.F., et. al., ^{67}Ga-citrate scanning in gastrointestinal malignancies. Clin. Nucl. Med. 3:179 1978.

465. Lentle B.C., Dierich H.C., Castor W.R., ^{67}Ga-citrate in detecting recurrent rectal carcinoma after abdomino-perineal resection. J. Can. Assoc. Radiol. 30:165 1979.

466. Kondo M., Hashimoto S., Kubo A., et. al., ^{67}Ga scanning in the evaluation of esophageal carcinoma. Radiology 131:723 1979.

467. Teates C.D., Preston D.F., Boyd C.M., Galium-67 citrate imaging in head and neck tumors: report of cooperative group. J. Nucl. Med. 21:622 1980.

468. Hoffer P.B., Pinskey S.M., Organ systems in which gallium-67 is of limited utility, in "Gallium-67 Imaging", P.B. Hoffer, C. Bekerman, R.E. Henkin, eds., John Wiley and Sons, New York, 1978, pp. 161-167.

469. Henkin R.E., Gallium-67 in the diagnosis of inflammatory disease, in "Gallium-67 Imaging", P.B. Hoffer, C. Bekerman, R.E. Henkin, eds., John Wiley and Sons, New York, 1978, pp. 65-92.

470. Hoffer P.B., Gallium and infection. J. Nucl. Med. 21:484 1980.

471. Hoffer P.B., Use of gallium-67 for detection of inflammatory diseases: a brief review of mechanisms and clinical applications. Int. J. Nucl. Med. Biol. 8:243 1981.

472. Staab E.V., McCartney W.H., Role of gallium-67 in inflammatory disease. Sem. Nucl. Med. 8:219 1978.

473. George E.A., Codd J.E., Newton W.T., et. al., Gallium-67 citrate in renal allograft rejection. Radiology 117:731 1975.

474. Thakur M.L., Coleman R.E., Welch M.J., Indium-111 labeled leukocytes for the localization of abscesses: preparation, analysis, tissue distribution and comparison with gallium-67 citrate in dogs. J. Lab. Clin. Med. 89:217 1977.

475. Sfakianakis G.N., Al-Sheikh J., Heal A., et. al., Comparison of scintigraphy with In-111 leukocytes and Ga-67 in the diagnosis of occult infection. J. Nucl. Med. 23:618 1982.

476. Heidenreich P., Kriegel H., Hor G., Biological investigation on the distribution of ^{67}Ga-citrate in rats in relation to sex and method of application. Int. J. Appl. Radiat. Isot. 25:557 1974.

477. Hayes R.L., Factors affecting uptake of radioactive agents by tumor and other tissues, in "Tumor localization with radioactive agents." IAEA, Vienna, 1976, pp. 29-45.

478. Otten J.A., Tyndall R.L., Estes P.C., et. al., Localization of gallium-67 during embryogenesis. Proc. Soc. Exp. Biol. 142:92 1973.

479. Tyndall R.L., Chaskes S.J., Carlton J.E., et. al., Gallium-67 distribution in pregnant mammals. J. Exp. Zool. 195:417 1976.

480. Newman R.A., Gallagher J.G., Clements J.P., et. al., Demonstration of Ga-67 localization in human placenta. J. Nucl. Med. 19:504 1978.

481. Winchell H.S., Sanchez P.D., Watanabe C.K., et. al., Visualization of tumors in humans using ^{67}Ga-citrate and the Anger whole body scanner, scintillation camera and tomographic scanner. J. Nucl. Med. 11:459 1970.

482. Larson S.M., Schall G.L., Gallium-67 concentration in human breast milk. J. Am. Med. Assoc. 218:257 1971.

483. Tobin R.E., Schneider P.B., Uptake of ^{67}Ga in the lactating breast and its persistance in milk. J. Nucl. Med. 17:1055 1976.

484. Fogh J., ^{67}Ga-accumulation in malignant tumors and in the prelactating or lactating breast. Proc. Soc. Exp. Biol. 138:1086 1971.

485. Paterson A.H., Yoxall A., Smith I., et. al., The concentration of ^{67}Ga and ^{45}Ca in the lactating mammary gland and its relevance to the tumor uptake of ^{67}Ga-citrate. Cancer Res. 36:452 1976.

486. Hayes R.L., Szymendera J.J., Byrd B.L., Effect of food intake on the tissue distribution of gallium-67. J. Nucl. Med. 20:938 1979.

487. Anghileri L.J., Prpic B., A new colloidal ^{68}Ga-compound for liver scanning. Int. J. Appl. Radiat. Isot. 18:734 1967.

488. Anghileri L.J., A new ^{68}Ga-compound for kidney scanning. Int. J. Appl. Radiat. Isot. 19:421 1968.

489. Goulet R.T., Shysh A., Noujaim A.A., Investigation of ^{68}Ga-tripolyphosphate as a potential bone-scanning agent. Int. J. Appl. Radiat. Isot. 26:195 1975.

490. Matté G.G., M.Sc. Thesis, University of Alberta, 1982.

491. Perkinson J.D., Eldridge J.S., Cooper B.M., Gallium chemistry of biological interest. Radiology 61:543 1953.

492. Swartzendruber D.C., Hubner K.F., Effect of external whole-body X-irradiation on gallium-67 retention in mouse tissues. Rad. Res. 55:457 1973.

493. Bradley W.P., Alderson P.O., Eckelman W.C., et. al., Decreased tumor uptake of gallium-67 by animals after whole-body irradiation. J. Nucl. Med. 19:204 1978.

494. Fletcher J.W., Herbig F.K., Donati R.M., ^{67}Ga citrate distribution following whole-body irradiation or chemotherapy. Radiology 117:709 1975.

495. Freeman C.R., Lisbona R., Palayew M., Lung scanning with ^{67}gallium citrate for detection of acute radiation changes. J. Can. Assoc. Radiol. 33:25 1982.

496. Richmand S.D., Levenson S.M., Bunn P.A., et. al., ^{67}Ga accumulation in pulmonary lesions associated with bleomycin toxicity. Cancer 36:1966 1975.

497. Bekerman C., Hoffer P.B., Bitran J.D., et. al., Gallium-67 citrate imaging studies of the lung. Sem. Nucl. Med. 10:286 1980.

498. Manning D.M., Strimlan C.V., Turbiner E.H., Early detection of busulfan lung. Clin. Nucl. Med. 5:412 1980.

499. Lentle B.C., Castor W.R., Khaliq A., et. al., The effect of contrast lymphangiography on localization of ^{67}Ga-citrate. J. Nucl. Med. 16:374 1975.

500. Shysh A., Mallet-Paret S., Lentle B.C., et. al., Altered biodistribution of radiogallium following BCG treatment in mice. Int. J. Nucl. Med. Biol. 8:349 1981.

501. Lentle B.C., Starrveld E., Presentation. Proc. Can. Assoc. Nucl. Med. 1981.

502. Tedesco F.J., Coleman R.E., Siegal B.A., Gallium citrate Ga-67 accumulation in pseudomenbranous colitis. JAMA 235:59 1976.

503. Ajmani S.K., Pircher F.J., Ga-67 citrate in gynecomastia. J. Nucl. Med. 19:560 1978.

504. Waxman A.D., Beldon J.R., Richli W., et. al., Steroid induced suppression of gallium uptake in tumors of the central nervous system. J. Nucl. Med. 19:480 1978.

505. Chilton H.M., Witcofski R.L., Watson N.E., et. al., Alteration of gallium-67 distribution in tumor-bearing mice following treatment with methotrexate. J. Nucl. Med. 22:1064 1981.

506. Kelson D.P., Alcock N., Yeh S., et. al, Pharmacokinetics of gallium nitrate in man. Cancer 46:2009 1980.

507. Donahue D.M., Leonard J.C., Basmadjian G.P., et. al., Thymic gallium-67 localization in pediatric patients on chemotherapy. J. Nucl. Med. 22:1043 1981.

508. Spencer R.P., Suresh P.L., Karimeddini M.K., Acquisition of thymic uptake of radiogallium in a child after therapy for infection. Clin. Nucl. Med. 7:407 1982.

509. Stepanas A.V., Maisey M.N., Hyperprolactinaemia as a cause of gallium-67 uptake in the breast. Br. J. Radiol. 49:379 1976.

510. Taylor A., Nelson H., Vasquez M., et. al., Renal gallium accumulation in rats with antibiotic-induced nephritis: clinical implication. J. Nucl. Med. 21:646 1980.

511. Linton A.L., Clark W.F., Driedger A.A., et. al., Acute interstitial nephritis due to drugs. Ann. Intern. Med. 93:735 1980.

512. Oster Z.H., Larson S.M., Wagner H.N., Possible enhancement of ^{67}Ga citrate imaging by iron dextran. J. Nucl. Med. 17:356 1976.

513. Hagan P.L., Halpern S.E., Stern P.H., The effect of certain variables on the tumor and tissue distribution of tracers. V. False carrier effect, II, Fe. Invest. Radiol. 17:53 1982.

514. Stern P.H., Halpern S.E., Hagan P.L., The effect of certain variables on the tumor and tissue distribution of tracers. II. False carrier effect, III, Fe. Invest. Radiol. 17:386 1982.

515. Hagan P.L., Halpern S.E., Stern P.H., The effect of certain variables on the tumor and tissue distribution of tracers. IV. False carriers: ferric citrate. Invest. Radiol. 16:229 1981.

516. Larson S.M., Rasey J.S., Grunbaum Z., et. al., Pharmacologic enhancement of gallium-67 tumor-to-blood ratios for EMT-6 sarcoma (BALB/c mice). Radiology 130:241 1979.

517. Scheffel U., Tsan M.F., Effect of serum unbound iron-binding capacity on the tissue distribution of ^{67}Ga in abscess-bearing rabbits. Nukl. Med. 19:274 1980.

518. Hayes R.L., Rafter J.J., Carlton J.E., et. al., Studies of the in vivo uptake of Ga-67 by an experimental abscess. J. Nucl. Med. 23:8 1982.

519. Sephton R., Martin J.J., Modification of distribution of gallium 67 in man by administration of iron. Br. J. Radiology 53:572 1980.

520. Sephton R., Martin J.J., ^{67}Ga imaging incorporating administration of iron. Int. J. Nucl. Med. Biol. 8:341 1981.

521. Smith F.W., Dendy P.P., Pocklington T., et. al., A preliminary investigation of ^{67}Ga citrate distribution in hyperferraemic patients. Eur. J. Nucl. Med. 5:327 1980.

522. Halpern S.E., Hagan P.L., Chauncey D., The effect of certain variables on the tumor and tissue distribution of tracers. I. Carrier. Invest. Radiol. 14:482 1979.

523. Brucer H.D., Hayes R.L., Perkinson J.D., Preliminary data on gallium-67. Radiology 61:602 1953.

524. Anghileri L.J., Thouvenot P., Brunotte F., et. al., Effects of various cations on the in-vivo distribution of ^{67}Ga citrate. Int. J. Nucl. Med. Biol. 9:195 1982.

525. Hoffer P.B., Samuel A., Bushberg J.T., et. al., Effect of desferoxamine on tissue and tumor retention of gallium-67. J. Nucl. Med. 20:248 1979.

526. Hoffer P.B., Samule A., Bushberg et. al., Desferoxamine mesylate (Desferal): a contrast-enhancing agent for gallium-67 imaging. Radiology 131:775 1979.

527. Oster Z.H., Som P., Sacker D.F., et. al., The effects of deferoxamine mesylate or gallium-67 distribution in normal and abscess-bearing animals. J. Nucl. Med. 21:421 1980.

528. Koizumi K., Tonami N., Hisada K., Deferoxamine mesylate enhancement of ^{67}Ga tumor-to-blood ratios and tumor imaging. Eur. J. Nucl. Med. 7:229 1982.

529. Oster Z.H., Som P., Sacker D.F., et. al., The use of chelating agents for the reduction of radiation dose. J. Nucl. Med. 22:P44 1981.

530. Saji H., Yokoyama A., Hata N., et. al., Enhancement of ^{67}Ga tumor-to-blood ratios by chelating agents. Radioisotopes 29:9 1979.

531. Moerlein S.M., Welch M.J., Raymond K.N., Use of tricatecholamide ligands to alter the biodistribution of gallium-67. J. Nucl. Med. 23:501 1982.

532. Carter J.E., Kwang G.J., Gallium accumulation in intramuscular injection sites. Clin. Nucl. Med. 4:304 1979.

533. Leonard J.C., Humphrey G.B., Vanhoutte J.J., Positive ^{67}Ga-citrate scans in patients receiving Corynebacterium parvium. Clin. Nucl. Med. 3:370 1978.

534. Sty J.R., Starshak R.J., Hubbard A.M., Ga-67 scintigraphy: calcium gluconate extravasation. Clin. Nucl. Med. 7:377 1982.

535. Forgacs P., Wahner H.W., Fey T.F., et. al., Gallium scanning in the detection of abdominal abscesses. Am. J. Med. 65:949 1979.

536. Newcomer A.D., Wahmer H.W., Gallium scan: clue to diagnosis of starch peritonitis. Clin. Nucl. Med. 4:465 1979.

537. Marlette J.M., Ma K.W., Shafer R.P., Effect of hemodialysis on ^{67}Ga-citrate scanning. Clin. Nucl. Med. 5:401 1980.

538. Hayes R.L., Carlton J.E., Byrd B.L., Localization of ^{67}Ga in soft tissue tumors in experimental animals. J. Nucl. Med. 10:406 1969.

539. Hayes R.L., Nelson B., Swartzendruber D.C., et. al., Gallium-67 localization in rat and mouse tumors. Science 167:289 1970.

540. Aulbert E., Disselhoff W., Sorje H., et. al., Lysosomal accumulation of ^{67}Ga-transferrin in malignant tumors in relation to their growth rate. Eur. J. Cancer 16:1217 1980.

541. Taylor D.M., Hammersley P.A., Tumor localization of gallium in relation to DNA synthesis, transferrin and lysosomal enzyme activity, in "Progress in Radiopharmacology", P.H. Cox, ed., Elsevier/North Holland Biomedical Press, New York, 1979, pp. 63-73.

542. Hammersley P.A., Taylor D.M., ^{67}Ga-citrate incorporation and DNA synthesis in tumors, in "Radiopharmaceuticals", G. Subramanian, B.A. Rhodes, J.F. Cooper, V.J. Sodd, eds., Soc. Nucl. Med. publ., New York, N.Y., 1975, pp. 447-451.

543. Okuyama S., Takeda S., Sato T., et. al., Biological mechanisms of gallium-67 tumor deposition. Sci. Rep. Res. Inst. Tohoku Univ. 26:22 1979.

544. Bichel P., Hansen H.H., The incorporation of ^{67}Ga in normal and malignant cells and its dependence on growth rate. Br. J. Radiol. 45:82 1972.

545. Merz T., Malmud L., McKusick K., et. al., The mechanism of ^{67}Ga association with lymphocytes. Cancer Res. 34:2495 1974.

546. Sephton R.G., Kraft N., ^{67}Ga and ^{59}Fe uptakes by cultured human lymphoblasts and lymphocytes. Cancer Res. 38:1213 1978.

547. Hammersley P.A., Cauchi M.N., Taylor D.M., Uptake of ^{67}Ga in the regenerating rat liver and its relationship to lysosomal enzyme activity. Cancer Res. 35:1154 1975.

548. Gullino P.M., The internal milieu of tumors. Prog. Exp. Tumor Res. 8:1 1966.

549. Hammersley P.A., Taylor D.M., Gallium-67 accumulation and the vascularity of tumors, in "Progress in Radiopharmacology", PH Cox, ed., Elsevier/North Holland Biomedical Press, New York, 1979, pp. 81-87.

550. Tzen K.Y., Oster Z.H., Wagner H.N., et. al., Role of iron-binding proteins and enhanced capillary permeability on the accumulation of gallium-67. J. Nucl. Med. 21:31 1980.

551. Rogers J.A., Noujaim A.A., Gallium-67 transport across artificial biological membranes. Int. J. Nucl. Med. Biol. 8:315 1981.

552. Vallabhajosula S.R., Harwig J.F., Wolf W., Effect of pH on tumor cell uptake of radiogallium in vitro and in vivo. Eur. J. Nucl. Med. 7:462 1982.

553. Orii H., Nakamura K., The mechanism of adsorption of Ga-67 citrate to cultured cells. Eur. J. Nucl. Med. 5:155 1980.

554. Weiner R.E., Schreiber G.J., Hoffer P.B., et. al., The relative stabilities of ^{67}Ga complexes of lactoferrin and transferrin at various pH's. Int. J. Nucl. Med. Biol. 8:371 1981.

555. Vallabhajosula S.R., Harwig J.F., Wolf W., The mechanism of tumor localization of gallium-67 citrate: role of transferrin binding and effect of tumor pH. Int. J. Nucl. Med. Biol. 8:363 1981.

556. Glickson J.D., Webb J., Gams R.A., Effects of buffers and pH on in vitro binding of ^{67}Ga by L1210 leukemic cells. Cancer Res. 34:2957 1974.

557. Hayes R.L., Chemistry and radiochemistry of metal-ion nuclides commonly employed in radiopharmaceuticals, in "The Chemistry of Radiopharmaceuticals", H.D. Heindel, H.D. Burns, T. Honda, L.W. Brady, eds., Masson publ., New York, 1978, pp. 155-168.

558. Muranaka A., Ito Y., Hashimoto M., et. al., Uptake and excretion of ^{67}Ga-citrate in malignant tumors and normal cells. Eur. J. Nucl. Med. 5:31 1980.

559. Burleson R.J., Johnson M.C., Head H., In vitro and in vivo labelling of rabbit blood leukocytes with ^{67}Ga-citrate. J. Nucl. Med. 15:98 1974.

560. Hammersley P.A., Taylor D.M., The mechanism of localization of ^{67}gallium citrate in experimental abscesses. Eur. J. Nucl. Med. 4:271 1979.

561. Gelrud L.G., Arseneau J.C., Milder M.S., et. al., The kinetics of ^{67}gallium incorporation into inflammatory lesions: experimental and clinical studies. J. Lab. Clin. Med. 83:489 1974.

562. Burleson R.L., Holman B.L., Tow D.E., Scintigraphic demonstration of abscesses with radioactive labelled leukocytes. Surg. Gynecol. Obstet. 141:379 1975.

563. Dhawan V.M., Sziklas J.J., Spencer R.P., Localization of Ga-67 in inflammatory lesions in the absence of circulating polymorphonuclear leukocytes. J. Nucl. Med. 19:292 1978.

564. Menon S., Wagner H.N., Tsan M.F., Studies on gallium accumulation in inflammatory lesions. II. Uptake by Staphylococcus aureus. J. Nucl. Med. 19:44 1978.

565. Emery T., Hoffer P.B., Siderophore-mediated mechanism of gallium uptake demonstrated in the microorganism Ustilago sphaerogena. J. Nucl. Med. 21:935 1980.

566. Tsan M.F., Studies on gallium accumulation in inflammatory lesions. II. Roles of polymorphonuclear leukocytes and bacteria. J. Nucl. Med. 19:492 1978.

567. Tsan M.F., Chen W.Y., Scheffel U., et. al., Studies on gallium accumulation in inflammatory lesions: I. Gallium uptake by human polymorphonuclear leukocytes. J. Nucl. Med. 19:36 1978.

568. Camargo E.E., Wagner H.N., Tsan M.F., Studies on gallium accumulation in inflammatory lesions. IV. Kinetics of accumulation and the role of polymorphonuclear leukocytes in the distribution of gallium in experimental inflammatory lesions. Nukl. Med. 18:147 1979.

569. Ostroy F., Johnston R.B., Gams R.A., Effects of hypoxia on ^{67}Ga incorporation into human granulocytes. Cancer Res. 39:4971 1971.

570. Robbins E.B., Cherou L.R., Blaufox M.D., Studies on the accumulation of radiopharmaceuticals in injured tissues, in "Radiopharmaceuticals II: Proceedings of the Second International Symposium on Radiopharmaceuticals", V.J. Sodd, D.R. Hoogland, R.D. Ice, eds., Society of Nuclear Medicine, New York, 1979, pp. 385-92.

571. Terner U.K., Wong H., Noujaim A.A., et. al., Differential uptake of ^{67}Ga in canine tumors and abscesses, in "Radiopharmaceuticals II: Proceedings of the Second International Symposium on Radiopharmaceuticals", V.J. Sodd, D.R. Allen, D.R. Hoogland, R.D. Ice, eds., Society of Nuclear Medicine, New York, 1979, pp. 309-319.

572. Anghileri L.J., ^{67}Ga-citrate accumulation by staphlococcal abscesses and by tumors - a common cause: changes in the alkaline earths metabolism. Nukl. Med. 13:151 1974.

573. Anghileri L.J., On the similarity of alkaline earths metabolism and ^{67}Ga accumulation by liver as revealed by acute thioacetamide intoxication. J. Nucl. Med. Biol. 19:145 1975.

574. Anghileri L.J., Studies on the relationship between ^{67}Ga-citrate accumulation and calcium metabolism in tumor cells. Nukl. Med. 12:257 1977.

575. Anghileri L.J., Heidbreder M., On the mechanism of accumulation of ^{67}Ga by tumors. Oncology 34:74 1977.

576. Anghileri L.J., Role of the tumor phospholipids in the accumulation of ^{67}Ga-citrate. J. Nucl. Biol. Med. 16:21 1972.

577. Anghileri L.J., Robert J., Isomorphous ionic replacement. Experimental evidence for the hypothesis proposed to explain gallium-67 accumulation. J. Nucl. Med. Allied Sci. 26:113 1982.

578. Haubold U., Aulbert E., $^{67}Gallium$ as a tumor scanning agent-clinical and physiological aspects, in "Medical Radioisotope Scintigraphy", Vol. 2, IAEA, Vienna, 1973, pp. 553-6.

579. Larson S.M., Rasey J.S., Allen D.R., et. al., A transferrin-mediated uptake of gallium-67 by EMT-6 sarcoma. II. Studies in vivo (BALB/c mice). J. Nucl. Med. 20:843 1979.

580. Sephton R.G., Harris A.W., Gallium-67 citrate uptake by cultured tumor cells, stimulated by serum transferrin. J. Natl. Cancer Inst. 54:7263 1975.

581. Harris A.W., Sephton R.G., Transferrin promotion of ^{67}Ga and ^{59}Fe uptake by cultured mouse myeloma cells. Cancer Res. 39:1213 1978.

582. Chen D.C., Newman E., Turkall R.M., et. al., Transferrin receptors and gallium-67 uptake in vitro. Eur. J. Nucl. Med. 7:536 1982.

583. Hayes R.L., Rafter J.J., Bryd B.L., et. al., Studies on the in vivo entry of Ga-67 into normal and malignant tissue. J. Nucl. Med. 22:325 1981.

584. Sephton R.G., De Abrew S., Hodgson G.S., Mechanisms of distribution of gallium 67 in mouse tumor hosts. Br. J. Radiol. 55:134 1982.

585. Sephton R.G., Relationships between the metabolism of ^{67}Ga and iron. Int. J. Nucl. Med. Biol. 8:323 1981.

586. Larson S.M., Rasey J.S., Allen D.R., et. al., Common pathway for tumor cell uptake of gallium-67 and iron via a transferrin receptor. J. Natl. Cancer Inst. 64:41 1980.

587. Larson S.M., Rasey J.S., Nelson N.J., et. al., The kinetics of uptake and macromolecular binding of Ga-67 and Fe-59 by the EMT-6 sarcoma-like tumor of BALB/c mice, in "Radiopharmaceuticals II: Proceedings of the Second International Symposium on Radiopharmaceuticals", V.J. Sodd, D.R. Allen, D.R. Hoogland, R.D. Ice, eds., Society of Nuclear Medicine, New York, 1979, pp. 297-308.

588. Morgan E.H., Appleton T.C., Autoradiographic localization of ^{125}I labelled transferrin in rabbit reticulocytes. Nature 223:1371 1969.

589. Hemmaplardh D., Morgan E.H., The role of endocytosis in transferrin uptake of reticulocytes and bone marrow cells. Br. J. Haematol. 36:85 1977.

590. Larson S.M., Factors determining tumor affinity for gallium-67 citrate, in "Radiopharmaceuticals Structure-Activity Relationships", R.P. Spencer, ed., Grune and Stratton Publisher, Toronto, 1981, pp. 167-181.

591. Noujaim A.A., Lentle B.C., Hill J.R., et. al., On the role of transferrin in the uptake of gallium by tumor cells. Int. J. Nucl. Med. Biol. 6:193 1979.

592. Terner U.K., Wong H., Nougaim A.A., et. al., ^{67}Ga and ^{59}Fe uptake in human melanoma cells. Int. J. Nucl. Med. Biol. 6:23 1979.

593. Wong H., Terner U.K., English D., et. al., The role of transferrin in the in vivo uptake of gallium-67 in a canine tumor. Int. J. Nucl. Med. Biol. 7:9 1980.

594. Verhoeff N.J., Noordeloos P.J., Binding of transferrin and uptake of iron by rat erythroid cells in vitro. Clin. Sci. Molec. Med. 52:87 1977.

595. Anghileri L.J., Thouvenot P., Brunotte F., et. al., In vitro and in vivo effects of ferric citrate on ^{67}Ga-citrate uptake by DS sarcoma tumors. Eur. J. Nucl. Med. 7:266 1982.

596. Gams B.A., Webb J., Glickson J.D., Serum inhibition of in vitro ^{67}Ga binding by L1210 leukemic cells. Cancer Res. 35:1422 1975.

597. Galbraith G.M., Galbraith R.M., Raulk W.P., Transferrin binding by human lymphoblastoid cell lines and other transformed cells. Cell. Immunol. 49:212 1980.

598. Kukushkin Y.N., Dkhara S.C., Preparation of a cis-diammine and tetra ammine of platinum (II). Russ. J. Inorg. Chem. 15:304 1970.

599. Dhara S.C., A rapid method for the synthesis of cis-$[Pt(NH_3)_2Cl_2]$. Ind. J. Chem. 8:193 1970.

600. Hoeschele J.D., The preparation, purification and analysis of cis-Pt $(NH_3)_2Cl_2$. Biophysics Dept., University of Michigan.

601. Hoeschele J.D., Butler T.A., Roberts J.A., Analysis and refinement of the microscale synthesis of the ^{195m}Pt-labeled antitumor drug, cis-dichlorodiammineplatinum (II), cis-DDP. Radiochim. Acta, in press.

602. Riabchikov D.I., The reactions with thiosulphate as a method for determining the nature of isomerism of platinum complex compounds. Compt. Rend. Acad. Sci. URSS 32:344 1941.

603. Basolo F., Lederer M., Ossicini L., et. al., A paper chromatographic study of some platinum (II) compounds. II. Separation of cis- and trans- dihalogenodiammineplatinum (II) complexes. J. Chrom. 10:262 1950.

604. Metzler C.M., Elfring C.M., McEwen A.S., A computer package of pharmacokinetic modelling. Biometrics 30:562 1974.

605. Sedman A.J., Wagner J.G., "AUTOAN, A decision-making pharmacokinetic program", The Upjohn Centre for Clinical Pharmacology, University of Michigan Medical Centre, Ann Arbour, Michigan, 1976.

606. Boxenbaum H.G., Riegelman S., Eloshoff R.M., Statistical estimations in pharmacokinetics. J. Pharmacokinet. Biopharm. 2:123 1974.

607. Sokal R.R., Rohlf F.J., "Biometry", W.H. Freeman and Co., San Francisco, 1969, pp. 127-206.

608. Klyuchnikov N.G., Saveleva R.N., The production of chloroplatinites by reduction of chloroplatinates with hydrazine hydrate and its salts. Russ. J. Inorg. Chem. 1:126 1956.

609. Vêges M.M., Nouveau mode de préparation du chloroplatinite de potassium. Bull. Soc. Chim. France 19:879 1898.

610. Hackh's Chemical Dictionary, J. Grant, ed., McGraw-Hill Book Company, Toronto, 1969.

611. Goremykin V.I., Hydrazine compounds of platinum and palladium. Compt. Rend. Acad. Sci. URSS 33:227 1941.

612. Basolo P.F., Pearson J.F., "The Chemistry of Platinum and Palladium". Wiley Interscience, New York, 1970, p. 468-90.

613. Lebedniski V.V., Golovnya V.A., Ammonia compounds of bivalent platinum. Chem. Abst. 44:5257 1950.

614. Chatt J., Gamlin G.A., Orgel L.E., The visible spectra of some platinous ammines. J. Chem. Soc. 486 1958.

615. Reishus J.W., Martin D.S., Cis-dichlorodiammineplatinum (II). Acid hydrolysis and isotopic exchange of the chloride ligands. J. Am. Chem. Soc. 83:2458 1961.

616. Ginzburg S.I., Ezerskaya N.A., Prokofeva I.V., et. al., "Analytical Chemistry of Platinum Metals", John Wiley and Sons, Toronto, 1975, pp. 167-407.

617. Haldur B.C., Chemical consequences of the (n, gamma) reaction on platinum compounds. J. Am. Chem. Soc. 76:4229 1954.

618. LeRoy A.F., Wehling M.L., Sponseller H.L., et. al., Analysis of platinum in biological materials by flameless atomic absorption spectrophotometry. Biochem. Med. 18:184 1977.

619. Pera M.F., Harder H.C., Analysis of platinum in biological materials by flameless atomic absorption spectrometry. Clin. Chem. 23:1245 1977.

620. Denniston M.L., Sternson L.A., Repta A.J., Analysis of total platinum derived from cisplatin in tissue. Anal. Lett. 14:451 1981.

621. Yudelevich I.G., Vall G.A., Torgov V.G., et. al., An extraction atomic absorption determination of gold in solution. Russ. J. Anal. Chem. 25:752 1970.

622. Gilbert E.N., Glukhova G.V., Glukhov G.G., et. al., Neutron activation determination of gold in rocks using dibutyl sulphide extraction for gold separation. J. Radioanal. Chem. 8:39 1971.

623. Torgov V.G., Khlebnikov A.A., Atomic absorption determination of gold in a flame and by means of a flameless graphite atomizer after preliminary extraction and separation with petroleum sulfides. Russ. J. Anal. Chem. 32:753 1977.

624. Solvsten S., Determination of gold in serum and urine by neutron activation analysis. Scand. J. Clin. Lab. Invest. 1:39 1964.

625. Gilbert E.N., Veriovkin G.V., Mikhailov V.A., et. al., Neutron activation determination of noble metals for the analysis of technological and natural materials. J. Radioanal. Chem. 38:147 1977.

626. El-Guebeily M.A., Rassoul A.A., Foriad H., Gamma spectrometric determination of the ^{199}Au to ^{198}Au ratio in pile irradiated gold targets. Radiochim. Acta 2:217 1964.

627. Sykes T.R., Stephens-Newsham L.G., Apps M.J., et. al., Assay of platinum and gold in biological material by neutron activation analysis. J. Radioanal. Chem. 69:441 1982.

628. Bennett J.V., Kirby W.M., A rapid, modified ultra-filtration method for determining serum protein binding and its application to new penicillins. J. Lab. Clin. Med. 66:721 1965.

629. Tsan M.F., Scheffel U., Tzen K.Y., et. al., Factors affecting the binding of gallium-67 in serum. Int. J. Nucl. Med. Biol. 7:270 1980.

630. Noujaim A.A., Terner U.K., Turner C.J., et. al., Alterations of gallium-67 uptake in tumors by cis-platinum. Int. J. Nucl. Med. Biol. 7:289 1981.

631. Bates G.W., Schlabach M.R., The nonspecific binding of Fe^{+3} to transferrin in the absence of synergistic anions. J. Biol. Chem. 250:2177 1975.

632. Katz S., Squire W., Metal ion-protein complex formation: buffer competitive effects. J. Theor. Biol. 82:405 1980.

633. Schlabach M.R., Bates G.W., The synergistic binding of anions and Fe^{+3} by transferrin. J. Biol. Chem. 250:2182 1975.

634. Williams R.J., Metal binding to biopolymers: some thoughts on antitumor activity. Rec. Results Cancer Res. 48:1 1974.

635. Stjernholm R., Warner F.W., Robinson J.W., et. al., Binding of platinum to human transferrin. Bioinorg. Chem. 9:277 1978.

636. Freireich E.J., Gehan E.A., Rall O.P., et. al., Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey and man. Cancer Chemother. Rep. 50:219 1966.

637. Terner U.K., Lentle B.C., Noujaim A.A., et. al., The effect of cis-platinum treatment on the in vivo distribution of gallium-67 in mice. Submitted for publication.

638. Thompson R.B., "A Short Textbook of Haematology", Pitman Medical Publ. Co., Toronto, 1979. p. 228.

639. Gardiner M.E., Morgan E.H., Effect of reduced atmospheric pressure and fasting on transferrin synthesis in the rat. Life Sciences 29:1641 1981.

640. Conrad M.E., Crosby W.H., Intestinal mucosal mechanisms controlling iron absorption. Blood 22:406 1963.

641. Warner F.W., Demannuelle M., Stjernholm R., et. al., Response of transferrin bound iron to treatment of rat lymphosarcoma with cis-dichlorodiammineplatinum (II). J. Clin. Hematol. Oncol. 7:18 1978.

642. Bradley W.P., Alderson P.O., Weiss J.F., Effect of iron deficiency on the distribution and tumor uptake of Ga-67 citrate in animals. J. Nucl. Med. 20:243 1979.

643. Flatt R.D., Carpenter A.B., Identification of crystalline material in urine of rabbits. Am. J. Vet. Res. 32:655 1971.